The Role of Nutrition and Estrogen on Holstein Calf Development, and Adenogenesis

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Abstract

Raising replacement heifers consumes a large portion of dairy and beef producers' income. An ultimate goal of producers is to decrease inputs and maximize outputs to produce fertile replacement heifers. Manipulating early postnatal growth and development through diet enables this practice to be successful. Puberty is greatly influenced by body weight (BW). Once heifers reach puberty, they become fertile and can achieve their reproductive potential. Growth can easily be influenced during the preweaning phase of development. Offering calves a higher plane of nutrition through milk diets prior to weaning hastens development and can lead to an earlier age at the onset of puberty. The objective of the first study was to understand how plane of nutrition influences BW, bone mineralization, and organ growth during the preweaning phase of development in Holstein calves. Calves offered a higher plane of nutrition experienced greater BW gains, increased bone mineralization, and accelerated organ growth. The second study evaluated plane of nutrition on reproductive development, specifically adenogenesis in Holstein heifer calves. Adenogenesis, or the development of uterine glands, is initiated in the early postnatal period. It involves rapid endometrial epithelial cell proliferation, germinal bud formation, invasion into the stroma, and extensive branching and coiling. Little is known about how nutrition impacts adenogenesis in ungulates, however, this study provided validation that it does drive gland formation. Additionally, this study assessed the influence of exogenous estradiol on reproductive development and adenogenesis when given after the completion of adenogenesis. We can confirm that exogenous estradiol given after the completion of adenogenesis does not alter the outcomes of gland formation. The beneficial effects of feeding a

higher plane of nutrition to calves prior to weaning on bone mineral density, organ growth, and adenogenesis may provide new possibilities for understanding the impacts of early nutrition on calf immune responses and productive lifespan of the cow. Collectively, these studies emphasize the importance of nutrition during preweaning growth and development of Holstein calves

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ADG	Average Daily Gain
BW	Body Weight
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BROMO	Bromocryptine Mesylate
BrdU	Bromodeoxyuridine
CP	Crude Protein
DES	Diethylstilbestrol
DEXA	Dual Energy X-ray Absorptiometry
DM	Dry Matter
ECW	Empty Carcass Weight
EGF	Epidermal Growth Factor
EB	Estradiol Benzoate
FGF	Fibroblast Growth Factor
FXD	Frizzled Receptors
GI	Gastrointestinal
HPN	High Plane of Nutrition
HGF	Hepatocyte Growth Factor
HH	Hip Height
IGF	Insulin-like Growth Factor
IGF-1R	Insulin-like Growth Factor Receptor
LPN	Low Plane of Nutrition
LE	Luminal Epithelium
MR	Milk Replacer
MY	Milk Yield
NOR	Norgestomet
P	Postnatal day
P4	Progesterone
PGF2α	Prostaglandin F2alpha
RLX	Relaxin
SBW	Scanned Body Weight
SCFA	Short Chain Fatty Acid
UGKO	Uterine Gland Knock Out
wk	Week
wt	Weight

Chapter 1

Literature Review

Management of replacement heifers involves decisions, practices, and knowledge that affect future productivity in both beef and dairy herds. Raising replacement herds requires the investment of time, feed, and adequate resources. Ideally, the aim of raising replacement heifers is to minimize inputs while maximizing outputs without limiting potential success and productivity (Hoffman and Funk, 1992). One of the largest expenses in the dairy and beef industries is producing and maintaining an acceptable replacement herd. The next generation of improved genetics lies in the selection of replacement heifers (Lamb, 2013) and it is vital that heifers are bred as yearlings and calve as two-year-olds to increase economic efficiency (Perry and Cushman, 2013).

A dairy herd consists of two major, economically demanding components; the milking herd and the replacement herd (Kristensen, 1992). The economic demand stems from many factors including culling rate of the milking herd, calving interval, mortality rate, age at first calving, live calving rate, and reproductive efficiency (Tozer and Heinrichs, 2001). Raising replacement heifers is second in cost, following feed cost for dairy producers (Bailey and Currin, 2009), but is necessary for the reproductive potential of a dairy herd.

A major focus for raising replacement heifers is the physiological processes that impact puberty. Puberty is influenced greatly by both age and weight and determines future reproductive success or failure (Funston et al., 2012). The most crucial input involves the feed costs to grow heifers with sufficient conditioning to reach puberty, breeding size, and calving size (Funston et al., 2012). According to Shamay et al. (2005), 0.7 kg/d of average daily gain (ADG) is most

advantageous for achieving greatest performance in Holstein dairy heifers (Shamay et al., 2005). Considering weight as a major influence of the reproductive timeline, it is important to understand the significance of preweaning nutrition, preweaning and prepubertal growth, and reproductive tract development.

Milk replacers (MR) are commonly used during preweaning growth stage in dairy calves, and the composition of MR undoubtedly influences the growth and development of young calves. In the past, producers fed what is often referred to as a 'conventional' MR diet to limit-feed MR and increase the onset and amount of concentrate feed consumption. This practice provides an economic relief in feed cost (Sejrsen and Purup, 1997). However, a more nutrient-rich MR designed for heightened growth rates, a diet often referred to as an accelerated MR diet, is now being investigated by numerous groups as an alternative to the conventional diet, especially when weight at puberty is an important consideration. Providing calves with a higher plane of nutrition may obstruct concentrate consumption but positively impacts growth and decreases the age at onset of puberty, conception, and calving. Understanding that a larger quantity of MR must be fed to reach sufficient calf growth, accelerated MR feeding strategies usually coincide with increased initial costs. Also, if excessive weight gain results in fat deposition, future milk production may be compromised because of reduced allometric mammary gland growth during the postnatal period (Sejrsen and Purup, 1997). Milk production results from successful breeding and is the major source of income for producers. It is important to understand the hormonal, functional, and structural elements of heifer calf reproductive development to enable producers to reach their target revenue.

Plane of nutrition impacts the onset of puberty. However, the impact of plane of nutrition on postnatal organ development, bone mineralization, and reproductive tract development is not

well understood. The role that nutrition plays on uterine development is especially important given that several key features of uterine development occur postnatally, and little is known about how nutrition influences uterine development. Postnatal development of uterine glands is especially concerning because uterine glands are critical components of establishing and maintaining pregnancies (Gray et al., 2001a; Filant and Spencer, 2014)

The goal of this thesis research is understanding the relationship between nutrition and postnatal organ growth, bone mineralization, and reproductive development while enhancing our understanding of the underlying mechanisms involved with health, longevity and pregnancy success of cattle. The specific aims are to 1) gain an understanding of how early postnatal nutrition influences growth, development, and health of neonatal calves, and 2) to understand how nutrition impacts reproductive development and specifically adenogenesis, in heifer calves. The literature review that follows will highlight early postnatal feeding strategies aimed to promote efficient calf growth, development, and potential productivity. It also will also overview uterine gland development and discuss the impacts of calf growth on reproductive performance.

Goals of Postnatal Calf Feeding Strategies

Producers strive to achieve efficient growth and health during the preweaning phase to maximize profitability (Garcia et al., 2015). Industry standards indicate dairy and beef calves should achieve puberty around 10 to 12 months of age so they may have acceptable fertility when bred at 13 to 15 months of age. This will target first calving around 2 years of age. Striving for calves with increased BW permits them to reach pubertal and breeding size at a younger age, decrease their age at first calving, and therefore, increase revenue corresponding to early onset of milk production once young heifers have calved (Rincker et al., 2011). Utilizing MR diets and

starter grain to manipulate preweaning growth is important to produce calves with ample conditioning. A milk-based product is essential to provide adequate protein, fat, and nutrients until calves can be sustained strictly on a grain or forage-based diet. It is important to optimize preweaning growth because too little gain may jeopardize the onset of puberty and lifelong fertility. Mechanisms of these outcomes will be discussed in further detail later in this chapter.

The optimal goal of beef replacement heifer growth is to reach approximately 60 to 65% of their expected mature BW by time of breeding (Patterson et al., 1992). This equates to an average of 1-1.5 lbs/d of gain from weaning to breeding (Patterson et al., 1992). Once beef heifers are determined pregnant, they should be offered rations enabling growth to reach 85% of their expected mature BW at fist calving (Lamb, 2013). Dairy heifer replacements should reach approximately 55% of mature BW at first conception and 85% of expected mature BW by first calving (National Research Council, 2001). Growth can be compromised if calves are offered suboptimal feed rations during postnatal development.

The 'transition' period is considered the time when a calf consumes grain and milk simultaneously (Drackley, 2008). Ideally, calves can be completely weaned from milk diets once they are consistently consuming 2 lbs of grain per day for a minimum of 3 days (NAHMS, 2007). Producers strive to push early consumption of starter grain in order to hasten development and provide an economic relief of feeding costs.

Early Postnatal Calf Growth

Postnatal calf growth includes an increase in body size and mass as well as modifications in the composition of tissues and organs (Heinrichs and Hargrove, 1987; Owens et al., 1993). Animal growth primarily focuses on BW, body stature, and lean muscle deposition (Owens et al.,

1993). As such, growth rates of young calves are typically evaluated by measuring birth weight, weekly BW, wither heights, and hip heights.

Beef Calves

Nutrient management and feed intake during the suckling phase of beef heifers are important factors for subsequent reproductive potential. Beef calves are kept with their dams during the suckling phase until weaning, and producers rely on the benefits of growth implants and creep feeding of calves for their impact on reproductive performance (Prichard et al., 1989). Growth implants are widely used during the suckling, growing, and finishing phases of beef cattle replacement development and typically contain an estrogen compound, a nonsteroidal compound with estrogenic activity, or a synthetic androgen (Patterson et al., 1992). Growth implants remain popular because they increase growth rate and feed efficiency, which decreases cost of raising replacement beef heifers (Staigmiller et al., 1983). Increased weaning weights dominate a large portion of revenue in the cow-calf production system (Patterson et al., 1992). To improve growth performance, producers will provide creep feed to their beef calves. Creep feeding is the practice of providing supplemental concentrate feed to the nursing animal in a structure that does not allow the cow to consume the feed which ensures additional weight gain of the calf (Lopes et al., 2014).

Dairy Calves

Traditional feeding practices in dairy calves have catered to a slower rate of gain compared to beef calves. One reason for this is management-related. In contrast to beef calves, dairy calves are separated from their dams at time of birth and then fed milk or a milk-replacer product along with starter grain for 6 to 8 weeks (NAHMS, 2007). Dairy calves are typically offered a milk product once or twice a day and the overall consumption of milk is, thereby reduced when compared with average suckling rates, which ranges from 7 to 10 suckling events

each day (Kmicikewycz et al., 2013). Economically, milk replacer and whole milk are consistently similar in price, ranging from $1.13-1.14 to $2.09, depending on the source (Karszes, 2007; Quigley, 2007). Feeding MR may seem more expensive because cash is paid out of pocket per unit, but nutrient intake of calves can more easily be managed when feeding MR versus whole milk. Crude protein and fat are typically found in higher concentrations in whole milk which may lead to accelerated rate of gain due to excess fat deposition (Sejrsen and Purup, 1997). Nutrient and cost break down of MR and whole milk are summarized in Figure 1-1 (Quigley, 2007).

Weaning and Post-Weaning Calf Growth

On average, producers wean calves from their milk diet to a concentrate diet at 8.4 weeks of age (Kehoe et al., 2007). There has been a lasting trend to wean calves earlier to cut down on feed and labor costs during the preweaning stages (Kehoe et al., 2007). Currently, 33.2% of producers wean their heifers at 8 weeks of age, 20.5% wean at 6 weeks of age, and only 5% wean at less than 4 weeks of age (the remaining percentages wean at varying times) (NAHMS, 2007). Time of weaning should be dependent on the amount of starter grain consumed per calf rather than age of calf (Corbett, 2007).

Weaning strategy is important to prevent loss of weight gain during the weaning period and allow calves a relaxed transition from a milk-based diet to a concentrate diet (Sweeney et al., 2010). Accelerated fed calves tend to experience a more stressful transition from their milk diet to a high concentrate diet than restrict fed calves, resulting in potential weight loss. Geiger et al., compared a control MR (22% CP: 20% fat) and an accelerated MR (27% CP: 10% fat) fed at 0.54 versus 0.85 kg of DM/day, respectively, and found that calves fed the accelerated treatment had greater preweaning BW, weaning weights, and preweaning average daily gains (ADG)

compared to control calves, but ADG during weaning decreased compared to control treated calves. However, no differences in starter grain intake or feed efficiencies [Feed Efficiency = Intake (kg)/ADG (kg/d)] were detected in this study (Geiger et al., 2014).

Regardless of feeding protocol, sudden weaning from milk can lead to distress and signs of hunger in calves (Sweeney et al., 2010). Sweeney et al. (2010), conducted a study looking at gradual weaning versus abrupt weaning. Results indicated that abruptly weaned calves that were consuming 9 kg/d of MR and inadequate starter grain, lost weight during the three days post-weaning. However, the calves weaned over a ten-day period, consuming the same quantities of MR, exhibited no weight loss due to increase starter intake (Sweeney et al., 2010). In a similar study done by Khan et al. (2007b), utilizing a 'step-down' weaning method resulted in an increased starter intake post-step and post-weaning when feeding MR at 20% of BW for the first 25 days and then gradually reducing to 10% of BW until weaning. Although there was decreased starter intake in the pre-step phase, calves were heavier and had greater rumen development at time of weaning than conventionally fed calves (Silper et al., 2014).

Post-weaning weight gain is important for calves to reach their full productive potential. Therefore, it is critical that producers incorporate a weaning strategy that minimizes weight loss at time of weaning. Heifers that grow faster will be younger at puberty, become productive sooner, and cost less in terms of feeding (Hill et al., 2008).

Strategies for Raising the Suckling Dairy Calf

Early postpartum nutrition and feed supply are crucial to the health and growth potential of young calves. Feeding colostrum, or the first secretions from the mammary glands after birth, from one hour after birth up to 12 hours after birth, is an important initial step when rearing dairy calves. Immunoglobins (IgG) found in colostrum provide calves with antibodies through

absorption into the small intestine. These IgG are necessary for passive immunity against microbial bacteria found in their environment (Kruse, 1983; Godden, 2008; Kryzer et al., 2015). Questions arise regarding which strategy of feeding colostrum is best for young calves. Various strategies exist to optimize colostrum feeding to achieve maximal performance. Included among them are: fresh frozen colostrum, fresh refrigerated colostrum, heat-treated in a pasteurizer then frozen, and heat-treated then frozen. The aim of altering colostrum handling is to reduce bacterial growth in colostrum that could potentially be harmful to health (Kryzer et al., 2015).

Immediately following colostrum feeding, calves are maintained on milk fom various sources. Prior to the development of milk replacers, producers fed milk from the bulk tank or waste milk from sick cows. Feeding nonpasteurized waste milk increases the incidence of ingesting pathogenic organisms. This practice can negatively impact the health of young dairy calves and can be economically insufficient (Otterby and Linn, 1981).

Since the development of MR, it is common for producers to feed synthetic milk or MR to their calves. Nutrient supply can be controlled through the use of MR, ensuring producers their calves are receiving adequate nutrients for growth, development, and health.

Traditional Schemes for Milk Feeding Dairy Calves

Prior to MR development, producers fed saleable or waste milk to their calves. Saleable milk comprises fresh or treated milk from cows or from the bulk tank. Nonsaleable waste milk comprises transition milk after calving, abnormal milk, or milk from cows being treated for illnesses (NAHMS, 2007).

Milk replacers were developed in the 1950-1960's. Today's calf MR diets contain two main ingredients; crude protein (CP) and crude fat. The quantity of these two ingredients drives the cost of MR. Normally, as fat concentration increases, protein concentrations decreases to offset the cost. An industry baseline for a conventional MR is now established at 20% CP: 20%

fat (Kertz, 2011), fed at 8 to 10% of BW/d (Sweeney et al., 2010). Variety, popularity, and quality of the protein and fat also influence price of MR (Otterby and Linn, 1981). Acceptable, yet costly protein sources include dried whey protein concentrate, dried skim milk, casein, dried whey, and dried whey product (Jones, May 2007). Supplementing MR diets with soy protein isolate, soy protein concentrates, and egg proteins provide an economic relief to the producer and are acceptable, non-animal proteins (Drackley et al., 2006). The most popular fat sources used in MR are from animal sources such as, butterfat, lard and tallow, because of their high digestibility. Due to public perception and consumer health concerns, there has been an increase in use of vegetable fat mixtures (Huuskonen et al., 2005). Consumer perception and its impacts on market prices force producers to seek more economically efficient MR rations or alter their preweaning feeding strategy (i.e. waste milk, whole milk, once-a-day feeding, early weaning, etc.) (Otterby and Linn, 1981).

Effects of Increasing Crude Protein and Fat Consumption

As will be discussed in the next section, a common practice is to limit-feed MR to accelerate calf starter consumption. However, doing so may reduce protein intake and lead to suboptimal increases in ADG, gain to feed ratio, and lean muscle deposition (Bartlett et al., 2006). In a study by Blome et al., increasing the CP in MR from 16 to 26% increased ADG, and lean tissue deposition (Blome et al., 2003). Bartlett and others performed a similar study and saw an increase in ADG, efficiencies of gain, and increased lean tissue deposition when increasing feeding rate and protein to energy ratios in MR diets (Bartlett et al., 2006).

During the 1970's, it was believed that higher fat levels in MR diets would increase the energy intake of calves. Fat levels then increased in MR from 10 or 12 to 20% which resulted in a more expensive MR product (Kertz, 2013). It was soon discovered that increasing the amount of fat in a MR diet would negatively influence ADG. Varying the amounts of fat in a MR diet

(14, 17, 20, or 23%) resulted in a reduction of starter intake, apparent digestibility of dry matter, fat, nonfiber carbohydrates, Ca, and P as fat increased with consistent amounts of CP/Mcal of metabolizable energy (ME). The decreased starter intake and digestibility resulted in lower ADG when fat was in higher concentrations (Hill et al., 2009). Therefore, fat is necessary to include in MR diets, however, levels of increasing concentrations are not justified.

Conventional Milk Replacers

Traditionally, producers feed a liquid milk ration in varying quantities to meet the nutritional needs of calves and reach appropriate weaning age suitable for their operation (Quigley et al., 2006). Currently, 85.9% of dairy calves in the United States are fed MR and the remaining 14% are either fed waste milk or whole (saleable) milk (NAHMS, 2007).

In the past few decades, research has focused on limit feeding MR to influence starter grain consumption (Raeth-Knight et al., 2009; Soberon et al., 2012). During the first few weeks of postnatal development, ruminants are 'pseudo-monogastric' or non-functional ruminants, where food is not ruminated but rather travels directly to the abomasum (true stomach) and intestines (Roth et al., 2009). Milk bypasses the rumen by way of the esophageal groove. It then enters the abomasum and is absorbed in the small intestine, providing glucose as the primary energy substrate (Khan et al., 2007a; Khan et al., 2007b). The reflex of the esophageal tube can remain active during the first 12 weeks of age (Quigley, 1997). Rumen development can be hastened by providing starter grain, specifically carbohydrates, which allows for an energy substrate shift from glucose to short-chained fatty acids (SCFA) (Khan et al., 2007a; Montoro et al., 2013). Once calves are able to utilize SCFA, microbial populations begin to colonize in the rumen and fermentation advances to allow more SCFA production, which helps establish ruminal epithelium (papillae). These events represent the core features of rumen fermentation and nutrient uptake (Quigley, 1997; Khan et al., 2007b; Montoro et al., 2013; Silper et al., 2014).

This "conventional" feeding program is considered cost effective (Soberon et al., 2012) because it facilitates an easy transition from liquid to solid feed, stimulates rumen development in neonatal calves and enables early weaning (Quigley et al., 2006; Khan et al., 2011). Conventional feeding programs incorporate feeding a MR consisting of 20-22% CP and 15-20% fat, reconstituted to 12.5% solids (w/v in water), along with *ad libidum* starter grain (Raeth-Knight et al., 2009). The average actual cost of this conventional feeding strategy is $2.65/kg/d (Rincker et al., 2011).

Conventional Milk Replacer versus Milk Diets

There is evidence that feeding saleable or nonsaleable milk provides greater economical and physiological benefits than feeding MR. Godden et al. (2005), estimated feeding pasteurized nonsaleable milk to calves at 1 to 2 days of age until weaning provided an economic relief of $0.69/calf per day versus feeding a conventional MR (Godden et al., 2005). Calves fed the conventional MR exhibit lower ADG, decreased weights at weaning, and a greater incidence of mortality when compared to calves fed pasteurized nonsaleable milk. Moallem et al. (2010), performed a similar study feeding calves ad libitum fresh whole milk or a conventional MR. Whole milk treated animals had heavier BW by 3.1 kg and greater ADG of 0.074 kg/d during the preweaning period. Also, long-term results reveal that calves fed whole milk were inseminated earlier and calved younger than calves fed conventional MR (Moallem et al., 2010).

Overfeeding

The mammary gland is made up of both parenchyma and stroma. The parenchyma contains the alveolar ducts that embed into the stroma, which contains blood vessels, lymphatics, and nerves (Ciocca et al., 1982). The developmental rate of mammary glands in heifer calves coincides with BW gain from birth to 3 months of age and again from 10 to 12 months of age to 3 months of gestation (Lohakare et al., 2012). Allometric growth, or development of mammary

growth at a faster rate than BW, of parenchyma tissues occurs between 3 and 9 months of age, and again from 3 months of gestation until calving (Lohakare et al., 2012; Freetly et al., 2014). During allometric growth, mammary gland development is easily influenced by nutrition. It is important to ensure that heifer calves do not over condition during the suckling phase because it can interfere with subsequent milk production and longevity (Holloway, 1973). There also is evidence that feeding whole milk is potentially detrimental to growth and subsequent productivity of calves, as adipose deposition was doubled in calves fed whole milk versus MR (Moallem et al., 2010). When ADG is ≥ 0.97 kg/d during the prepubertal period, milk production is compromised during the first lactation. This results from reduced parenchyma growth in the mammary gland (Shamay et al., 2005; Krpalkova et al., 2014). There was an increase in luminal and epithelial structures within the parenchyma of heifers fed 950 g/d versus 650 g/d (Daniels et al., 2009). When feeding heifers differing nutrient intakes, heifers fed a greater nutrient diet had increased mammary fat pad weight, decreased parenchyma weight, and fewer parenchymal cell numbers (Meyer et al., 2006). The increase in fat pad weight, in response to elevated nutrient intake, is due to an increase in lipid retention and DNA content. The decreased parenchyma weight demonstrates that parenchymal tissues are refractory to adipocyte endocrine signaling (Meyer et al., 2006).

Accelerated Calf Feeding Programs

Although the initial nutritional cost of a conventional MR feeding strategy appear more economical, many operations are shifting away from this practice for fear of issues with low BW at weaning, slower growth rates, and an increase in mortality and morbidity (Khan et al., 2011). Several alternative strategies are now available for producers. With the continued goal of breeding heifers as young as physiologically acceptable, producers make all efforts in increasing early growth and development by manipulating postnatal diets. Offering calves increased

quantity and quality of MR has been termed as an accelerated or intensified MR feeding strategy. An accelerated MR regimen typically consists of 25-28% CP (generally from whey proteins) and 10-25% fat (generally from animal sources; tallow, lard, etc.), reconstituted at 12.5-17.5% (w/v in water), along with starter grain offered *ad libidum* (Cowles et al., 2006; Raeth-Knight et al., 2009). The average cost, calculated over a two-year period, of feeding an accelerated MR and a starter grain is $3.08/kg/d (Rincker et al., 2011). This creates a monetary increase in feeding heifer calves of $0.43/kg/d.

Numerous studies have compared conventional versus accelerated MR calf feeding strategies. To follow is a highlight of several prominent MR-feeding strategies. Cowles et al. (2006), performed a study feeding a conventional (20% CP: 20% fat) MR and an accelerated (28% CP: 20% fat) MR. Their results revealed that calves fed the accelerated MR had heavier BW, increased ADG, higher gain to feed ratios, and were taller prior to weaning. However, calves fed the conventional diet consumed more starter grain prior to weaning and during weaning (Cowles et al., 2006). Kmicikewycz et al. (2013), performed a similar study looking at control versus accelerated feeding along with frequency of feeding (two or four times/daily) (Kmicikewycz et al., 2013). Calves fed the accelerated diet experienced greater ADG and gain:feed ratios, regardless of frequency of feeding. Accelerated calves also had a greater heart girth, increased muscle total lipid, and there was no impact of diet or feeding frequency on health of the calves (Kmicikewycz et al., 2013). Feeding calves a higher plane of nutrition ensure that the energy consumed is met for lean muscle gain, tissue growth, and efficient BW gains (Bartlett et al., 2006) rather than fat storage (Hill et al., 2009).

Impacts of Early Postnatal Feeding Programs on Health

Several studies suggest that accelerated MR feeding strategies may have adverse effects on preweaning calf health but will positively influence innate immunity and immunization

responses after weaning. Calves fed a high plane of nutrition have a greater incidence of preweaning scours and respiratory scores (nasal and eye discharge, cough, etc.) (Raeth-Knight et al., 2009; Hengst et al., 2012). Neutrophil mRNA expression of two specific genes were activated, likely relating to the greater incidence of scours and respiratory issues; however, vaccination response was not compromised (Hengst et al., 2012). In another study, Holstein and Jersey calves fed a higher plane of nutrition had increased neutrophil oxidative bursts when co-cultured with *E. coli*. The greater activation of neutrophils in both studies suggests a greater innate immune response during postnatal growth of calves fed a higher plane of nutrition (Ballou, 2012).

Impacts of Early Postnatal Feeding Programs on Organ Development

The organs of young calves are developed at parturition, but little research has examined progressive growth of organs during early stages of life. In a study done by Hill et al. (2008), the trachea, heart, and lungs of accelerated fed calves were smaller in proportion to BW than conventional fed, but the livers and kidneys tended to be larger ($P < 0.07$ versus 0.09, respectively) (Hill et al., 2008). Similarly, Bartlett et al. (2006), reported heavier livers, hearts, and kidneys in heifers fed 1.75% of body versus heifers fed 1.25% of their BW (Bartlett et al., 2006). More work is needed to better understand how conventional and accelerated MR strategies impact early postnatal organ development, and how any changes in growth trajectory of these organs impacts animal growth, health, and lifelong productivity.

Impacts of Early Postnatal Feeding Programs on the Onset of Puberty

Puberty can be defined as the ability to reproduce successfully through fertilization. It is initiated by hormonal signals from the brain to the gonads (ovaries in a female, testis in the male) (Senger, 2005). As for bovine reproduction, female puberty is the stage in development in which

estrus is expressed and ovulation occurs (Patterson et al., 1992). Estrus is considered the time in which a female experiences 'heat', or receptivity to a male for reproduction.

Early preweaning-feeding practices, like conventional or accelerated MR programs, have been linked to reproductive performance of calves in later life. Underfeeding heifers during periods of critical growth can delay the onset of estrus (Otterby and Linn, 1981). Conversely, rapid growth of calves fed an accelerated MR diet will typically reach breeding size at a younger age, deliver their first calf earlier, and be more reproductively efficient through their lifetime (Rincker et al., 2011). In one study, heifers fed an accelerated MR diet were 31 days younger and 20 kg lighter at the onset of puberty than control-fed calves (Rincker et al., 2011). Van Amburgh et al. performed a study looking at prepubertal ADG and its correlation to age at first calving (Van Amburgh et al., 1998). Heifers that gained 0.68 kg/d calved at 24.5 months while those that gained 0.83 kg/d calved at 22.0 months and heifers that gained an average of 0.94 kg/d calved at 21.2 months of age (Van Amburgh et al., 1998).

Impacts of Early Postnatal Feeding Programs on First Lactation Milk Production

There are reports of reduced first lactation milk yield (MY) in calves fed accelerated MR diets (Harrison et al., 1983). Harrison et al., reported that rearing heifer calves on a high, moderate, or low rate of gain influenced mammary development within the first year of life (Harrison et al., 1983). Heifers reared on a low or moderate rate of gain had heavier mammary parenchyma, more ductal tissue and less mammary fat than animals reared at a high rate of gain. These data suggest that mammary development is impaired if heifers are reared on a high rate of gain (Harrison et al., 1983). Similar findings raise caution to implementing an accelerated MR feeding system. However, in a study done Rincker et al., heifers fed an accelerated diet tended to have a greater MY when genetic variation was removed from the equation and parent average milk values were used as a covariate (Rincker et al., 2011). In a similar study, Raeth-Knight et al.

conducted a study evaluating the effects of a conventional or accelerated MR feeding system on heifer performance (Raeth-Knight et al., 2009). Endpoints of prime interest included age at first calving and first lactation performance. Heifers fed an accelerated, high solids, high feeding (IHSHF) MR had their first calf 27.5 days earlier than calves fed a conventional MR; however, no difference was detected in first lactation performance (Raeth-Knight et al., 2009).

One also must consider age at first calving when examining first lactation milk production. Since most mammary development occurs prior to calving, an earlier age at first calving can alter milk production due to insufficient development of the mammary glands (Hoffman, 1997). However, increasing protein and energy intakes of calves during early stages of life can increase mammary development and in turn, improve MY at first lactation (Bar-Peled et al., 1997). In a recent study, when calves from two separate herds were offered more nutrients through their MR diet, every 1 kg of preweaning ADG resulted in an average of 850 kg of more MY at first lactation in one herd and 1,113 kg of more first lactation MY in the other herd (Soberon et al., 2012). This increase in first lactation MY accounts for 22% of the variation in MY between preweaning diets (Soberon et al., 2012). Similarly, when calves were offered diets with greater nutrient content or higher nutrient content and added amino acids, calves produced a greater amount of milk at first lactation then calves fed strictly whole milk (Margerison et al., 2013). Increasing preweaning growth rates may not have adverse effects on mammary development because from birth to three months of age, mammary development occurs at the same rate of development as BW, or isometric growth rate (Lohakare et al., 2012). It is the time at which allometric growth of the mammary gland that increased nutrients and BW gain can be harmful to mammary development (Lohakare et al., 2012).

Although the relationship between nutrition and first lactation performance remains disputable, there is evidence that lifetime cow performance is improved with accelerated calf feeding. Heifer calves that were fed to reach an ADG of 1.0 kg spent 120 days open versus heifers that were fed to reach 0.8 kg of gain per day spent 135 days open during their first postpartum anestrous period (Van Amburgh et al., 1998). Also, the second lactation milk yield was unaffected by treatment (Van Amburgh et al., 1998). Little data include cow performance beyond first lactation or 305-day mature equivalent (ME). Data that exist for first lactation and lifetime milk production encourage implementation of an accelerated MR diet. If used appropriately, this regimen could improve MY and increase milk revenue for producers.

Reproductive Tract Development

A long-term goal of rearing replacement heifers is to obtain maximal fertility rates. From time of birth to puberty, producers may lose or cull a number of heifer calves due to mortality or infertility (Nor et al., 2015). Despite the elevated cost, producers tend to keep all of their newborn heifer calves in order to alleviate uncertainty in availability of heifer calves at time of breeding (Nor et al., 2015). It is not only important to understand the production strategies that influence puberty, age at first calving, and first lactation milk yield, but also to understand the underlying mechanisms that govern reproductive potential and fertility.

Prenatal Reproductive Tract Development

Like most other organs, reproductive organs form during embryonic and fetal development. However, reproductive organs are unique given that they do not become completely functional until puberty (Murashima et al., 2015). The genetic sex of the gonads determines the specific development and determination of mammalian sex (Capel, 2000). The *Sry* gene, located on the Y chromosome, is known as the sex-determining gene and will initiate

development of testis rather than an ovary (Capel, 2000). Therefore, an XY embryo will become a male and an XX embryo will become a female (Spencer et al., 2012). In the presence of an XY embryo, the differentiated testis will secrete Müllerian-inhibiting substance along with other hormones that will regress the Müllerian ducts and progress the Wolffian ducts (Nef and Parada, 2000). However, in the presence of an XX embryo, the bipotential gonad will form an ovary due to the absence of the Y chromosome (Spencer et al., 2005).

The female reproductive tract development stems from the Müllerian ducts during prenatal organogenesis (Kobayashi and Behringer, 2003). The Müllerian ducts develop into the infundibula, oviducts, uterus, cervix, and anterior vagina during the prenatal stages of life in mammals (Gray et al., 2001a; Spencer et al., 2012). Prenatal organogenesis of the male reproductive tract systems forms from the Wolffian ducts (Spencer et al., 2012). The Wolffian ducts differentiate into the vas deferens, seminal vesicles, and epididymis (Nef and Parada, 2000). Succeeding growth and development of the sexually differentiated reproductive organs involves several signaling pathways and sex hormones (Murashima et al., 2015).

PGC Migration and The Initiation of Folliculogenesis

Primordial germ cells (PGC) aid in sexual differentiation through formation of the gonadal ridge in utero (Marion and Gier, 1971). PGC have been identified in the bovine yolk sac as early as day 18 (Wrobel and Suss, 1998). As the bovine embryo continues to elongate and develop into an embryonic body, PGC migrate from the hind and mid-gut portion of the yolk sac to the developing gonadal ridge (McNatty et al., 2000). The gonads remain sexually undifferentiated until 39 to 41 days of development and then will become ovaries or testis (Marion and Gier, 1971; Wrobel and Suss, 1998).

At this point in the ovary, the epithelial cords make up the ovarian medulla and cortex by day 45 in the bovine fetus (Marion and Gier, 1971; McNatty et al., 2000). By day 60, primordial

follicles exist and contain an immature oocyte (Lundy et al., 1999; Lin et al., 2002; Aerts and Bols, 2010). In bovine, the number of developing primordial follicles will persist until four to six years of life. The number of primordial follicles that develop will continuously decrease for the length of the animal's life (Erickson, 1966). Folliculogenesis is the process by which small primordial follicles progress to antral follicles capable of ovulation or follicles that undergo atresia (Picton, 2001). During the prepubertal stages, many follicles are present on the ovary in varying stages of development but do not ovulate (Marion and Gier, 1971). As puberty approaches, the duration of follicle waves is longer and mature follicles grow to reach ovulatory size (Bergfeld et al., 1994).

Uterine Development

Prenatal Uterine Development

In the female, the site at which the Müllerian duct crosses the gubernaculum, or the ovarian ligament, designates the uterotubal junction (Spencer et al., 2005). The oviduct originates from the anterior and lateral crossings of the duct whereas the uterus, cervix, and anterior vagina originate from the medial and posterior segments of the duct (Cunha, 1975). The degree to which the Müllerian ducts fuse determines the characteristics of the adult uteri (Spencer et al., 2005). Ruminant females develop a moderate-length, bicornuate uterus from the Müllerian ducts, comprised of one cervix and a short uterine body, along with other sex-specific organs (Gray et al., 2001a; Cooke et al., 2013). Specifically, the uterus consists of three functional layers, the endometrium, myometrium, and perimetrium which are histologically distinguishable by day 70 of gestation (Marion and Gier, 1971). The endometrium houses the luminal and glandular epithelium in stromal compartments, blood vessels, and raised, aglandular nodules that are predestined to become caruncles and can be seen by embryonic day 90. Similarly, by embryonic day 135 until birth, compact invaginations of luminal epithelium can be

observed to represent bud formation of glandular epithelium (Wiley et al., 1987). The myometrium is made up of an inner circular smooth muscle layer and an outer longitudinal smooth muscle layer. The outer, serous membrane of the perimetrium provides protection to the uterus (Gray et al., 2001a; Cooke et al., 2013). Although most work completed for prenatal uterine development involves the mouse model, it can be assumed that development is similar across species (Gray et al., 2001a).

Postnatal Uterine Development

The reproductive tract is the last major organ system to mature in mammals (Ramaley, 1979), and maturity is not fully complete until puberty. Most of the female reproductive tract organs are completely developed and differentiated at birth, but the uterus is not fully developed until 2 to 3 months after birth in mammals examined (Spencer et al., 2012). Specific tissues within the uterus cannot be histologically differentiated until after birth (Cooke et al., 2013).

Complete, postnatal uterine development involves three important events: 1. organization and stratification of the endometrial stroma; 2. myometrial, smooth-muscle growth and differentiation, and; 3. development of endometrial glands (Gray et al., 2001a; Cooke et al., 2013). The endometrium lines the uterine wall and encompasses the myometrium's two layers of smooth muscle, the inner circular and outer longitudinal layers (Spencer et al., 2005). Numerous aglandular caruncles and intercaruncular regions are located within the endometrium of the uterus (Atkinson et al., 1984). The caruncular regions form placentomes with the development of the placental cotyledons and serve as the fetal-maternal gas exchange during gestation (Wooding, 1992). The intercaruncular regions house countless numbers of uterine glands responsible for synthesizing, transporting, and secreting substances necessary for survival of a growing embryo (Filant and Spencer, 2014).

The uterus, the organ of pregnancy, has several essential functions related to reproduction (Spencer et al., 2005). It controls estrous cyclicity, primarily through its production of prostaglandin F2α (PGF2α), the luteolytic factor that initiates the end of the estrous cycle. Similarly, PGF2α is also is required for stimulation of uterine contractions through the myometrium to deliver the fetus during parturition (Senger, 2005). The uterus is the site for transport, storage, and maturation of spermatozoa prior to fertilization. Once fertilization occurs, the uterus is the site of embryonic attachment and fetal development throughout gestation (Bartol et al., 1999; Senger, 2005; Spencer et al., 2005).

Adenogenesis

Adenogenesis, or uterine gland development, is initiated at birth and is completed after 3 weeks of age in mice and 8 weeks of age in pigs and sheep (Gray et al., 2001a). Once complete, a cross-section of the uterine epithelium will contain hundreds of highly coiled, tubular-shaped glands that often extend from the uterine lumen deep into the endometrial stroma (Filant and Spencer, 2014). Limited information about the ontogeny of adenogenesis is available for cattle. This is likely due to the costly price of raising replacement heifers and the fact that the bovine model is much larger compared to other livestock species. It can be assumed that the bovine model is similar to the ovine model because uterine gland morphogenesis is exceedingly similar across several species, including laboratory rodents, domestic animals, and humans (Bartol et al., 1999). This section will describe adenogenesis by examining the well-studied sheep model.

Uterine glands are vital for the synthesis, secretion, and transport of enzymes, growth factors, cytokines, lymphokines, hormones, proteins, and other substances into the uterine lumen (Bazer, 1975; Gray et al., 2001a). Such secretions impact the conceptus, placental growth and fetal survival and development (Bazer, 1975). The uterine gland secretions exchanged during earlier pregnancy act as the conceptus' nutritional supply containing a variety of carbohydrates,

proteins, and lipids (Hempstock et al., 2004). Cytokines and growth hormones are associated with regulating placental development, trophoblast differentiation, implantation, and cytotrophoblast proliferation (Hempstock et al., 2004). A separate group of transport proteins and glycoproteins act as antioxidants, immune defenses, and immunosuppressors by inhibiting T-cells for the growing fetus (Rachmilewitz et al., 1999; Hempstock et al., 2004). Together, uterine gland secretions ensure the survival, health, and development of a growing conceptus and aid in the growth and expansion of the placenta during pregnancy.

Timeline of Adenogenesis

In sheep, adenogenesis begins on postnatal day (P) 0. The first week of postnatal development is characterized by glandular epithelial (GE) bud formation and the initial invagination and invasion from the luminal epithelium (LE) into the stroma between the intercaruncular sections (Taylor et al., 2000). From P7 to P21, the incipient buds undergo cell division, or 'tubulogenesis,' into the stroma, forming tube-like structures that will begin to coil outward, or towards the myometrium. Between P21 and P56, the tubular, coiled glands undergo branching morphogenesis, an event characterized by tubular and coiled glands forming terminal ends, or 'bud' structures deep into the stroma (Gray et al., 2001a; Cooke et al., 2013). Adenogenesis is completed at P56, or at the end of week 8 in sheep (Gray et al., 2001a) when caruncular and intercaruncular endometrial areas are histologically indistinguishable from adult uteri (Cooke et al., 2013). The developmental events are summarized in Figure 1-2.

Hormonal and Steroidal Influences on the Timing of Adenogenesis

The initial activation of adenogenesis is ovary-, adrenal-, and steroid- independent in sheep (Spencer et al., 2012). Initially, estrogens (E_2) were thought to mediate early phases of postnatal uterine development (Cooke et al., 2013). However, it now is clear that estrogens do not control the initial events of adenogenesis in sheep (Spencer et al., 2012). Neonatal ewe

uterine growth and adenogenesis were not affected by administration of a non-steroidal aromatase inhibitor, CGS 20267, which prevents the conversion of testosterone to E_2 (Carpenter et al., 2003a). Also, circulating E_2 levels were unchanged from birth to P56. Lastly, adenogenesis was unaffected on P14 when ewes were ovariectomized on P0 (Bartol et al., 1988b; Carpenter et al., 2003a). With that said, the involvement of estrogens with adenogenesis is species-dependent. For example, in pigs, administration of an anti-estrogen at birth inhibits adenogenesis, suggesting its necessity in this species (Cooke et al., 2013).

Adenogenesis is regulated through 'cross-talk', or interactions, between epithelial and stromal cells (Gray et al., 2001a; Spencer et al., 2012). Tissue environments during critical spatial and temporal phases of development are required to maintain changes in cell behavior, such as migration and proliferation that are necessary for gland development (Bartol et al., 1999). When these stromal and epithelial interactions are interrupted, epithelial morphogenesis and stromal organization and development will be altered or discontinued (Bartol et al., 1999; Gray et al., 2001a). These assertions have been supported by the creation of a uterine gland-deficient, or uterine gland knock out (UGKO) phenotype, simply by extending progesterone support into the postnatal period.

Progestins and glucocorticoids are known to suppress morphogenetic uterine changes (Ogasawara et al., 1983). It has been hypothesized that the elevated levels of progesterone (P4) during gestation and the increasing concentrations of glucocorticoids during parturition create an inactive state of the endometrium until birth (Bartol et al., 1988a). Progesterone during gestation inhibits adenogenesis by providing an anti-proliferative environment in the uterus; therefore, removal of the P4 dominant environment at birth initiates an endocrine cue for the activation of adenogenesis in the neonatal ewe (Gray et al., 2000; Cooke et al., 2013). This theory has been

repeatedly tested in ewes through continuous exposure of progestins following birth. Simulating a 'pseudo' gestational state with P4 will continuously inhibit adenogenesis; presumably through disruption of epithelial-stromal interactions (Bartol et al., 1999). Brief exposure of Norgestomet (NOR), a progesterone derivative, from birth to P13 inhibited adenogenesis. When the P4 block was removed, gland morphogenesis resumed, but the glands were underdeveloped and abnormal (Bartol et al., 1988a). Exposing neonatal ewes to NOR from birth until 8, 16, or 32 wk of age results in either a total absence of glands or slight, infrequent invaginations into the stroma that cannot be classified as glands (Gray et al., 2000). Long-term exposure of NOR coincides with diminished expression of ERα in the uterine epithelium (Gray et al., 2000a) and altered phenotype of LE and GE cells. Norgestomet must be used as the progestin analogue because it acts through P4 receptors whereas a pure P4 would be metabolized.

Exposing sheep to NOR, or other progestin derivatives, during early postnatal development impacts the uterus, and specifically gland number and shape, but has no appreciable effects on the brain, ovary, cervix, or vagina (Gray et al., 2000a). Seeing that NOR exposure only targets endometrium served as the foundation for developing the UGKO (Cooke et al., 2013). Ewes that have been exposed to NOR for as little as 8 wk were sufficient to generate a UGKO phenotype (Gray et al., 2000). Uterine gland knockout models have been utilized to study the specific actions or factors that influence uterine adenogenesis. Several UGKO studies identified reductions in ERα, prostaglandin receptor (PGR) expression, $FGFR2_{IIIb}$ (FGF receptor), and hepatocyte growth factor (HGF) (Gray et al., 2000; Gray et al., 2000a). Sheep expressing the UGKO phenotype have normal patterns of P4, E_2, and oxytocin receptor expression but experience altered estrous cycles. This is due to the lack of luteolytic pulses of PGF2α, which is produced by the LE. UGKO ewes can, however, respond to exogenous PGF2α (Gray et al.,

2000b). Pregnancy is also interrupted in ewes lacking uterine glands. Interestingly, fertilization and early embryonic development proceeds normally, but conceptus elongation and implantation do not occur (Gray et al., 2001b). With a lack of glands, the conceptus is not receiving adequate nutrients, growth factors, and enzymes, etc., from gland secretions, needed to support growth and development.

The importance of estrogen receptor alpha (ERα) for completion of adenogenesis has been established in sheep. Postnatal uterine development is associated with ERα that is expressed in the glandular epithelium and endometrial stroma as it proliferates and develops (Gray et al., 2001a). At P0-1 in the ewe, there is an increase in ERα expression, likely due to the immediate decrease in circulating progestins in the neonatal ewe (Gray et al., 2000a). Estrogen receptor alpha in stromal cells regulates uterine epithelial proliferation, specifically branching morphogenesis during P14 through P56, in response to estrogen or locally produced growth factors (Tarleton et al., 1999). Neonatal ewe exposure to EM-800, an antagonist of ERα, resulted in no impact on initial adenogenesis, and uterine growth, but coiling and branching morphogenesis were delayed (Carpenter et al., 2003a; Cooke et al., 2013). Again, actions of ERα are species-dependent. In pigs, ERα antagonist treatment at birth led to an inhibition of gland development by P7 because of an increase in stromal cell density. Treatment of the ERα from P7 to P13 did not inhibit gland penetration depth but did reduce endometrial thickness through altered organization of stromal cells (Tarleton et al., 1999). Concluding, disruption of ERα in porcine will result in improper endometrial development (Tarleton et al., 1999).

It is interesting to consider the pre-pubertal ovaries as drivers of adenogenesis. The removal of ovine ovaries at birth or P7 does not impact circulating E2 levels, (Carpenter et al., 2003a) although reduction in uterine weights resulted at P28 (Kennedy et al., 1974). Ontogeny of

adenogenesis was unaffected at P14 (Bartol et al., 1988b) but limited gland coiling and branching thereafter (Carpenter et al., 2003c). These observations implicate ovaries as being essential for adenogenesis, albeit not during the initial stages of the process. The specific ovarian-derived factors involved with these processes have not been defined (Carpenter et al., 2003d).

WNT genes are important regulators of uterine growth and influence adenogenesis from birth until the end of branching morphogenesis (Hayashi and Spencer, 2006). WNT genes direct cell and tissue growth and differentiation during gland morphogenesis in varying organs (Hayashi and Spencer, 2006). They perform in an autocrine or paracrine manner by associating with their frizzled (FZD) receptors or low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6), located on the extracellular surface (Dale, 1998). Several different WNT genes are expressed in either the luminal, stromal, or GE during the early postnatal period. WNTs 5A, 7A, and 11 are expressed in the LE from birth through subsequent gland development. WNT7A expression is associated with transformations of uterine morphogenesis whereas WNTs 5A and 11 regulate endometrial development and adenogenesis specifically (Hayashi and Spencer, 2006). WNT2B is localized only in the stroma from P14-P56 but can act on the stroma, LE, and GE (Spencer et al., 2005). Expression of WNT genes is altered by estradiol. Down regulation of expression of WNT2B, WNT7A, and WNT11 has been associated with altered adenogenesis through estrogen-induced disruption of endometrial adenogenesis (Spencer et al., 2012). Altered expression of WNT4, 7A, and 11 were detected after administration from birth to P5 of synthetic estrogen diethylstilbestrol (DES), which inhibits adenogenesis and uterine development (Hayashi et al., 2011). Studies have reported that uterine defects seen in DES-treated females might be a result of the down regulation of WNT7A expression (Spencer et al., 2012). The WNT7A-null

mouse exhibits malformations in the reproductive tract such as, alterations in patterning, void of uterine glands, deficient in stroma, and the smooth muscle is unorganized (Miller et al., 1998).

There are several stromal and epithelial-derived growth factors that likely influence epithelial proliferation, differentiation, and branching morphogenesis. Fibroblast growth factors (FGFs) and hepatocyte growth factor (HGF) are of central importance for epithelial and stroma development, including gland development and villous projections in several organs. FGF10 stimulates epithelial cell proliferation and differentiation through a paracrine manner, where stroma-derived FGF10 acts through FGF receptors to mediate epithelial development and function (Spencer et al., 2012). Bellusci et al. reported evidence of increased expression of FGF10 in a localized manner during early stages of lung development in the mouse model, suggesting FGF10 importance through branching morphogenesis (Bellusci et al., 1997). HGF behaves in a paracrine manner during the mesenchymal-epithelial interactions regulating mitogenic and morphogenic behaviors in developing liver, lung and mammary tissues (Weidner et al., 1993). FGF7 and its receptor $FGFR2_{IIIb}$ are expressed in the uterus from P1 to P56 whereas FGF10, HGF, and its receptor, MET, are expressed from P21 through P56 when branching morphogenesis is occurring (Spencer et al., 2012). Neonatal ewes exposed to improper levels of E_2 or P4 during early prenatal growth resulted in irregular patterns of growth hormone expression (Hayashi et al., 2004).

Both IGF-I and IGF-II are implicated as regulators of cell proliferation and differentiation during adenogenesis in sheep. Expression of IGF-I and IGF-II localizes in the endometrial stroma, and its receptor (IGF-1 receptor (IGF-1R)) is expressed in GE (Taylor et al., 2001). IGF production is influenced by E_2, and IGF-1 is known to activate ERα (Taylor et al., 2001; Spencer et al., 2005). Treatment of neonatal ewes with EM-800, an ERα antagonist, led to inhibition of

ERα activation through IGF (Carpenter et al., 2003a). Similarly, IGF-1 and IGF-11 expression were reduced in neonatal ewes exposed to improper E_2 (Spencer et al., 2005). This suggests that IGF-I and IGF-II are involved with postnatal adenogenesis and growth, specifically endometrial gland coiling and branching (Smith, 1998).

Prolactin (PRL) is a versatile hormone known in various organs to regulate growth and differentiation through extracellular matrix signaling (Lelievre et al., 1996). There is compelling evidence that PRL is an important component of normal adenogenesis. Its circulating concentrations are greater for the first few weeks after birth (Gray et al., 2001a). Glandular epithelium of branched uterine glands contain PRL receptors (PRL-R) in early postnatal and adult ewes (Gray et al., 2001a). When neonatal ewes were treated from P0-56 with a bromocryptine mesylate (BROMO) pellet, a PRL secretion inhibitor, their uteri contained fewer glands compared to control animals (Carpenter et al., 2003b). Also, when neonatal ewes were treated from P0-56 with a recombinant ovine PRL, their uteri contained a greater gland number and gland density on P14 and P56 (Carpenter et al., 2003b).

Lastly, recent evidence suggests that there are lactocrine factors associated with adenogenesis (Bartol et al., 2008). Colostrum provides a mechanism to transfer bioactive factors from mother to offspring, so they may act on the anterior pituitary, digestive tract, and the immune system. These bioactive factors are defined as lactocrine signaling from milk (Yan et al., 2006). Studies concluded in porcine have shown that fetal development of the female reproductive tract tissues were influenced by lactocrine-signaling through relaxin (RLX), a milk-borne bioactive factor. Neonatal pigs that consumed colostrum had altered ERα than pigs that consumed MR in absence of colostrum (Chen et al., 2011). Similarly, gilts had greater uterine weights and increased expression of ERα when exogenous RLX was given at birth (Chen et al.,

2011). From these observations, it was proposed that bioactive factors in colostrum could aid in developing tissues of neonates through delivery into the system from nursing (Yan et al., 2008). Similar studies on RLX have not yet been reported in sheep or cattle. However, bovine colostrum contains high concentrations of IGF-1 (500 μg/L), which is stable enough to withstand gastric acid environments. It is known to increase the growth-promoting activity of growth hormone that can regulate gut growth and function (Playford et al., 2000).

Exogenous Hormonal Manipulations of Adenogenesis

With the increasing use of E_2 implants for growth in the beef industry, there is a concern that exogenous E_2 during early postnatal development can adversely impact reproductive performance, and specifically uterine and gland development (Bartol et al., 1995). Certainly, beginning exogenous steroid treatments in the early postnatal period likely compromises uterine gland development. Bartol et al. administered P4 and estradiol benzoate (EB), a synthetic E_2, to beef heifers at three different time points (P0, P21, or P45) to define the resulting impact on adult uterine morphology. Heifers treated with P4 and EB at all times experienced reduced uterocervical weights, myometrial area, and endometrial area. There was also a reduction in gland density by 65, 22, and 33% for treated heifers on P0, P21, or P45, respectively (Bartol et al., 1995). Similarly, Carpenter et al. demonstrated that exposure of ewe lambs to estradiol-17β valerate (EV), an ERα agonist, beginning at birth, disrupts uterine development and adenogenesis (Carpenter et al., 2003a). Little work has been done looking at the affects of steroid implants on reproductive outcomes when given later in life. When implants were given during gestation, at approximately 100 days post breeding, calf birth and weaning weights were not affected but calving ease was inversely affected (Anthony et al., 1981). It can be assumed that steroid implants given beyond completion of adenogenesis will not have adverse affects on gland genesis, morphology, and function.

Summary

Management of replacement heifers in the dairy and beef industries involves strategic decisions, practices, and knowledge that affect future productivity. Early postnatal growth and development are essential for successful productivity and longevity of calves in dairy and beef herds. Utilizing the ability to manipulate calf growth through nutritional adjustment is an ongoing area of interest for researchers and producers. It has been established that nutrition impacts pre- and post-weaning, and pre- and post- pubertal growth of young calves. Several studies have reported the influence of under- and over-nutrition during the gestational period of dams and its impacts on fetal, survival, growth, and development. However, little research exists on the importance of nutrition and its influence on reproductive development, specifically adenogenesis, in young calves. Adenogenesis is a key component of survival, health, and development of a growing fetus. Much of the work done involving adenogenesis is performed in mice, pigs, and sheep with little being known about adenogenesis specific to cattle. This lack of research hinders progressive movement towards understanding the exact timeline of adenogenesis, along with the hormonal, steroidal, and cellular regulators of gland development specific to the bovine species.

Past research has stressed the need to better understand physiological processes and growth of neonatal calves so that producers can manipulate calf development to improve their selection criteria for replacement heifers. The overall focus of the following work is to provide the premise for studying nutrition and steroid influences on growth and reproductive development of neonatal Holstein calves. This focus will heighten our understanding of the relationship between nutrition and the physiological development Holstein calves. Specifically, the aims of this thesis were completed to:

1) Examine bone mineralization and organ development in calves fed different planes of nutrition, and

2) Examine adenogenesis in calves receiving exogenous estradiol while being fed different planes of nutrition.

Table 1-1: Nutrient and cost breakdown of MR versus WM. [Adapted from (Quigley, 2007)]

Item	Milk Replacer	Whole Milk
Amount fed, lb/day	1.25	9.52
DM, lb/day	1.19	1.19
CP, lb/day	0.25	0.30
Fat, lb/day	0.25	0.33
Cost, $/day	1.13	1.14

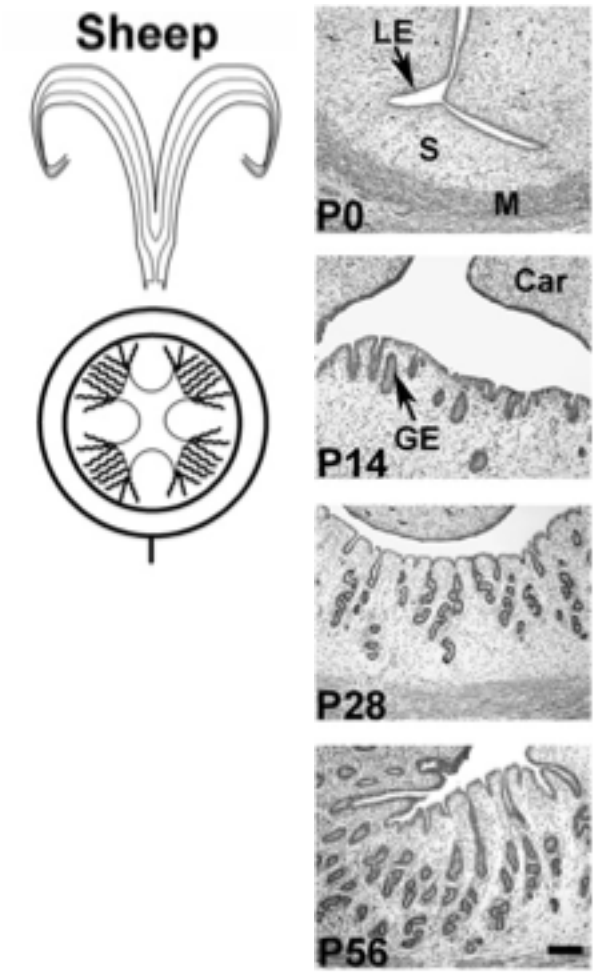

Figure 1-2: Ovine Uterus and timeline of Adenogenesis Top Left- Frontal section of medium-length bicornuate uterus in sheep. Bottom Left- Radial patterns of the uterine wall of sheep. Right- Uterine wall histoarchitectural development in sheep [Figure taken from (Spencer et al., 2012)].

Chapter 2

Increasing Plane of Nutrition Positively Impacts Holstein Bull Calf Growth, Bone Density and Organ Development

Introduction

Achieving sufficient growth and health of young calves is a major priority of dairy producers. Traditionally, producers limit feed MR to 8-10 % BW which hastens starter grain intake and reduces feed costs during the preweaning period (Soberon et al., 2012). This feeding strategy impairs calf growth potential during the early postnatal period (Brown et al., 2005). However, offering calves a higher plane of nutrition through MR diets during the preweaning period can lead to accelerated growth rates, increased average daily gain, improved feed efficiency, and lean muscle deposition (Bartlett et al., 2006). Using diets to manipulate growth during critical periods of development in young calves increases calf productivity and has long-term economic benefits for producers (Rincker et al., 2011).

The immune system of young calves is undeveloped and easily compromised during early postnatal development (Garcia et al., 2015). Lowering the risk of disease and mortality is an important management practice for producers. Early postnatal diet plays a large role in the successful growth and health of young calves. Studies suggest that offering calves a high plane of nutrition (HPN) may compromise calf health; however, when calves were offered a HPN they had greater neutrophil mRNA expression and equivalent vaccination responses compared to calves fed a LPN (Ballou, 2012; Hengst et al., 2012). The greater activation of neutrophils suggests a greater innate immune response during postnatal growth. Although the mechanism is unknown, it is clear that diet likely influences immune response and health of young calves.

Most organs are fully developed at parturition in young calves and other mammals (Hill et al., 2008). Very few studies exist analyzing normal growth rates of all organs and what influences this growth during early postnatal development in calves. Heavier livers, hearts, and kidneys, adjusted to BW, have been reported in studies when calves were offered a HPN versus a low plane of nutrition (LPN) through MR diets during the preweaning period (Bartlett et al., 2006; Hill et al., 2008). It is evident that diet likely influences the growth rates of several vital organs. Diet also influences bone health, specifically bone mineral density (Tucker et al., 1999). Although no reports indicate the importance of early postnatal diet and its influence on bone mineral density in calves, it has been hypothesized in humans that protein can have an affect on bone mineral density (Tucker et al., 1999). Knowing that growth is substantial after birth in young claves (Bartlett et al., 2006), it is beneficial to understand the significant influences of bone growth, specifically. This experiment examined how plane of nutrition impacts Holstein calf BW, height, bone mineralization, and organ size.

Materials and Methods

Animals and Experimental Design

Animal experiments were completed in accordance with and with the approval of the Virginia Tech Animal Care and Use Committee.

Holstein bull calves (n = 27) were fed 2 L fresh colostrum within 12 h after birth. A second colostrum feeding was completed within 8 h of the first feeding. Calves were housed in individual pens (167.64 cm x 121.92 cm) within a single curtain-sided, enclosed barn at the Virginia Tech Dairy Center (Blacksburg, Virginia). Pens were bedded with sawdust and the barn was fan-ventilated. The study was completed between March and June.

At birth, calves were assigned randomly to MR diets and time blocks. The two milk replacer diets included a LPN MR (n = 13) (Southern States Calf Maker MP; 20% CP and 20%

fat; Richmond, VA) fed at 441 g dry matter (DM) for the entirety of the study, and a HPN MR (n = 14) (Cow's Match, Warm Front; 27% CP and 10% fat; Land O'Lakes Animal Milk Products Co; Saint Paul, MN) fed at 882 g of DM during wk (wk) 1 and at 1431 g of DM in wk 2 through 8. Both MR diets were reconstituted to 15% [w/v in water] and fed twice daily using a peach teat, single calf feeder at 0600 and 1500 h. Water was offered *ad libitum* throughout the study. At the end of wk 2, starter grain (Southern States Intensity 22% Textured Calf Starter, Medicated) was offered to all calves at 1% of BW. Refusals were recorded daily at 0600 h. BW and hip height (HH) were recorded for all calves at birth and weekly during the study. If visual signs of scouring occurred, calves were offered oral electrolytes (Sav-A-Caf Electrolytes Plus, Milk Products, Chilton, WI) between feedings until diarrhea ceased. If scours was not resolved by the administration of oral electrolytes for two feedings along with MR, veterinarian attention was sought (HPN: n = 4; LPN: n = 3). Calves experiencing a fever of 103°F received Excenel subcutaneously (Zoetis, Florham Park, NJ; 2.2 mg/kg = 1.75 cc/ 100lbs) once daily for three days. Two calves were removed from the study after these symptoms were not resolved following veterinary care.

At the assigned time (2, 4, or 8 wk), calves were fasted for 18 h and euthanized via a lethal dose of barbiturate (Beuthanasia-D, Merck Animal Health, Millsboro, DE; 11.1 mg/kg).

Dual Energy X-ray Absorptiometry

Immediately following euthanasia a Dual Energy X-ray Absorptiometry (DEXA) scan was completed to analyze bone mineralization and body composition. Scans were performed using a GE Lunar Prodigy Advance Axial DEXA Densitometer (GE Medical Systems, Madison, WI.) compact bed (201cm (W) X 109cm (D) X 128cm (H); US/CALA version – encore v15). A high resolution, slow scan (scan mode: standard- 1.8 μGy) of the hind legs were used to quantify bone mineral density (BMD) and bone mineral content (BMC). A low resolution, fast scan (scan

mode: standard-0.4 μGy), excluding the head, was used to examine lean body mass and fat body mass. Scanned body weights (SCW) were generated from the DEXA scan automatically to calculate lean body mass and fat body mass.

Organ and Tissue Collections

Immediately following DEXA scanning, non-clotted blood was removed via carotid artery puncture. Each calf was then dissected to retrieve the heart, liver, lungs, pancreas, thymus, spleen, and kidneys. Kidneys were evaluated before and after perirenal fat was excised. All organs were rinsed, dried, and weighed. Small tissue samples (0.5 to 1 cm^3) were snap frozen in liquid nitrogen and stored at -80°C for future analysis. After removal of all internal organs, the digestive, respiratory and reproductive tracts were removed, and an empty carcass weight (ECW) was recorded.

Statistical Analysis

Growth, DEXA, and organ weight data were analyzed using the general linear model of the Statistical Analysis System (SAS institute, 1999). BW and HH data were analyzed using the PROC GLIMMEX procedure of SAS for repeated measures using initial birth weight as a covariate. Data presented as LSmeans and SEM. Significance determined at $P < 0.05$.

Results

Calf Growth

The ingredients and composition of both MR and the starter grain are shown in Table 2-1. Diets were not intended to be isocaloric, nor were they adjusted relative to BW. Rather, a fixed quantity of MR was fed to calves in each dietary treatment group. Total nutrient intake of milk replacer and calf starter are shown in Table 2-2. The occurrence of scours was similar between the LPN and the HPN MR diets. However, an increased incidence of veterinary attention was sought for calves fed the HPN MR diet due to noticeable rectal discomfort and fever.

Both diet ($P < 0.01$) and wk ($P < 0.01$) affected BW over the 8 wk period (Fig 2-1). Moreover, a diet by wk interaction was detected ($P < 0.01$), and within-wk comparisons determined that BW was greater ($P < 0.05$) in calves fed the HPN than those fed the LPN beginning at wk 3 (Fig. 2-1). HH was affected by diet ($P < 0.01$), wk ($P < 0.01$) and diet by wk interaction ($P < 0.05$). HH was increased ($P<0.05$) in HPN calves beginning at wk 2 (Fig. 2-1).

Average daily gain (ADG), BW, and HH were analyzed at 2, 4 and 8 wk of age (Fig. 2-2). Both diet ($P < 0.01$) and wk ($P < 0.01$) impacted cumulative ADG (Fig. 2-2). No diet by wk interaction was observed (Fig. 2-2). Final BW was affected by diet ($P < 0.01$) and wk ($P < 0.01$), and a diet by wk interaction ($P < 0.01$) (Fig. 2-2). The individual comparisons indicate that BW increased at a slower pace for LPN calves than HPN calves. Both diet ($P < 0.01$) and wk ($P < 0.01$) impacted HH at final time points 2, 4, and 8 wk (Fig. 2-2). Similarly, there was a diet by wk interaction ($P < 0.01$). Individual comparisons show the LPN fed calves at 2 wk were shorter than HPN fed calves ($P < 0.05$). At 4 wk, HH was not significantly different for calves fed differing diets but there was a significant difference noted at 8 wk between MR diets ($P < 0.05$) (Fig. 2-2).

DEXA Results

Scanned body weight (SBW; excluding the head) was impacted by diet ($P < 0.01$), wk ($P < 0.01$). A diet by wk interaction also was detected ($P < 0.01$) (Table 2-3). Lean content (kg; the sum of water, protein, and soft tissue mineral and glycogen) was influenced by diet ($P < 0.01$), wk ($P < 0.01$), and a diet by wk interaction ($P < 0.01$) (Table 2-3). There was greater ($P < 0.05$) lean content (kg) from 2, 4, and 8 wk HPN fed calves. Percent lean (of SBW) was not significantly different across diet, wk, or diet by wk (Table 2-3). Fat content (g; sum of lipid mass) was influenced by diet ($P < 0.01$), wk ($p < 0.01$), and diet by wk ($P < 0.01$) (Table 2-3). There were recognizable increases ($P < 0.05$) in fat content from 2, 4, and 8 wk HPN-fed calves.

Percent fat (of SBW) was affected by diet ($P < 0.05$), with HPN calves being greater than LPN calves, but no wk or diet by wk interaction was detected (Table 2-3).

Bone mineral content, measured in grams, was also impacted by diet ($P < 0.01$), wk ($P < 0.01$), and a diet by wk interaction ($P < 0.01$) (Table 2-3). At 2 wk, BMC (g) was not different between MR diets. Following that, BMC was greater ($P < 0.05$) in HPN-fed calves than LPN-fed calves. There was a significant increase in BMC from 4 wk to 8 wk seen in the HPN-fed calves ($P < 0.05$). When analyzing BMC as a percent of BW, there was no significant difference in diet, wk, or a diet by wk interaction. Bone mineral density was impacted by diet ($P < 0.01$), wk ($P < 0.05$), and diet by wk ($P < 0.05$) (Table 2-3). At 2 wk, BMD was not different between MR diets, but thereafter BMD was greater ($P < 0.05$) in HPN-fed calves than LPN-fed calves. Also, an increase ($P<0.05$) in BMD was detected in HPN calves at wk 8 when compared with wk 4.

Organ Weights

Absolute weights of the liver, heart, kidney, lungs, spleen, and thymus were affected by diet ($P < 0.01$), wk ($P < 0.01$), and a diet by wk interaction ($P < 0.01$). Weights (g) of the pancreas and perirenal fat were affected by diet ($P < 0.01$), but not by wk or by their interactions (Table 2-4). At 2 wk, spleen weights were not different between MR diets, but HPN calves contained greater ($P < 0.05$) spleen weights than LPN calves at 4 and 8 wk. No detectable difference in thymus weight were observed between MR diets at 2 wk, but thymus weights were greater ($P < 0.05$) in HPN-fed calves than LPN-fed calves at 4 and 8 wk. Gastrointestinal (GI) tract weights (g) were influenced by diet ($P < 0.01$) and wk ($P < 0.01$) but there was no diet by wk interaction detected.

Diet ($P < 0.01$), wk ($P < 0.01$), and the diet by wk interaction ($P < 0.01$) impacted ECW. A greater ($P < 0.05$) ECW was detected HPN calves than LPN calves at each time point. The greatest ($P < 0.01$) increase in ECW was detected at 8 wk in calves fed the HPN.

When organs were analyzed as a percentage of ECW, the spleen and thymus were impacted by diet ($P < 0.01$) and wk ($P < 0.01$). Diet by wk interactions ($P < 0.01$) also were detected, and in both cases these organs were most dramatically different between HPN and LPN calves at 8 wk. Adjusted liver weights were affected by diet ($P < 0.01$) and a diet by wk interaction ($P < 0.05$) but not by wk alone. At 4 wks, HPN fed calves had greater liver weights than LPN fed calves. Adjusted kidney weight, perirenal fat, and lungs were all impacted by diet ($P < 0.05$), with heavier organs detected in HPN fed calves with lungs being the only exception. However, this impact was not seen by wk or in a diet by wk interaction. Adjusted heart and pancreas weights were not affected by diet, wk, or diet by wk.

Discussion

This work confirmed that an increased % and amount of CP and energy content in a preweaning MR diet would result in different growth trajectories of young Holstein bull calves over an 8 wk period. There is a clear deviation in BW, HH, and ADG at 8 wks. These results are similar to numerous studies that have been executed to verify the growth benefits of offering calves a HPN versus LPN (Bartlett et al., 2006; Rincker et al., 2011; Soberon et al., 2012). Starter grain was only offered at 1% of total BW in this study, and this likely prevented calves in the LPN diet from achieving greater increases in BW like they would if they were offered *ad libitum* starter grain. In a study preformed by Davis Rincker et al.(2011), Holstein heifer calves experienced similar growth rates to the bull calves used in this study when offered a HPN versus LPN along with *ad libitum* starter grain (Rincker et al., 2011). The study was designed to examine bone and organ development in calves under different planes of nutrition, and these diets achieved that goal.

We can infer that the increased quantity of milk and high nutrient content of the HPN MR was the reason for the increased occurrence of diarrhea and need for vet attention. More vet

visits occurred in the HPN group. The incidence of these visits is similar to those reported elsewhere (Brown et al., 2005; Rincker et al., 2011).

Few studies exist investigating the differences in body composition of calves utilizing the DEXA scanner. The HPN fed calves had greater lean tissue mass and fat mass compared to the LPN fed calves. This can be expected as several reports indicate the increased lean muscle deposition with increased CP offered in the preweaning milk replacer diet (Blome et al., 2003; Bartlett et al., 2006; Rincker et al., 2011). Also, it can be predicted that HPN fed calves will have a greater fat mass because they are greater by volume than LPN fed calves. However, no visible adipose deposition was observed during the harvesting process. Typically, calves are consuming net energy for maintenance and gain for organs, bone, and muscle; and in turn they will not deposit fat during preweaning stages of life (Owens et al., 1993).

During the high resolution, slow scan, only the two hind limbs posterior of the pelvic bone were analyzed to determine BMC and BMD. The relative similarity in infantile growth trajectories and BW was indistinguishable at 2 wk. Once the HPN calves undergo exceptional growth by 4 and 8 wk, BMC and BMD were significantly different. At an increased rate of gain, relative growth rates of various tissues, including bone, will form from head to tail and from extremities to core (Owens et al., 1993). This could influence the increased BMC and BMD detected in HPN fed calves at 4 and 8 wk of age.

All vital organs are developed at birth. Little is known about the growth trajectories of organs during early stages of growth and if preweaning diets can hasten growth of organs. Results of this study are concurrent with Bartlett et al., where they saw heavier livers, hearts, and kidneys in heifers fed a HPN versus LPN (Bartlett et al., 2006). Results of this study show heavier livers, hearts, kidneys, lungs, spleen, and thymus indicating that nutrition may positively

influence vital organ growth trajectory. Interestingly, spleen and thymus weights increased significantly for HPN fed calves 8 wks. This may suggest that HPN fed calves could have a stronger or more responsive immune system due to the influence of adequate protein and energy supply during preweaning growth and development.

Deviations in growth rates, bone mineralization, and organ weights were evident between calves fed HPN and LPN diets. These outcomes are consistent with the contention that HPN feeding accelerates growth and improves health in young calves. The beneficial effects of HPN on bone mineral density and immune-centric organs provide new possibilities understanding the impacts of early nutrition on calf immune responses and productive lifespan of the cow.

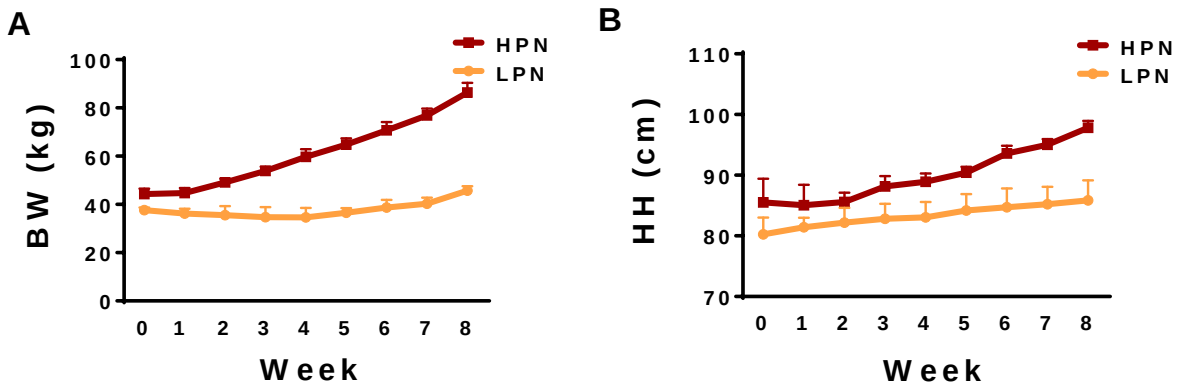

Figure 2-1. Growth parameters of bull calves fed LPN (low plane of nutrition) or HPN (high plane of nutrition) diets: Panel A: Weekly body weight (BW) of bull calves from birth and continuing weekly until 8 wk of age. Panel B: Weekly hip height (HH) of bull calves from birth and continuing weekly until 8 wk of age. The asterisks (*) indicate differences in within-wk comparisons of each diet ($P < 0.05$).

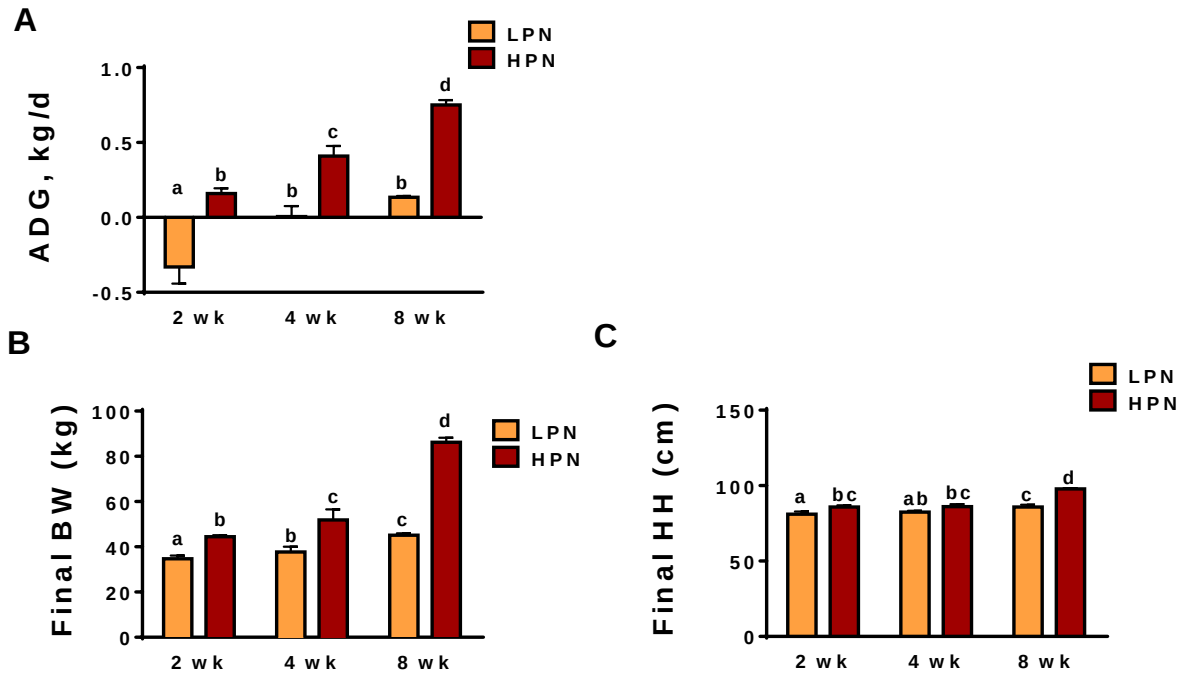

Figure 2-2. Final growth performances of Holstein bull calves fed LPN (low plane of nutrition) or HPN (high plane of nutrition) diets for 2, 4, 8 wks. Panel A. Average daily gain (ADG) (kg/d). Average daily gain = final BW – initial BW/ numbers of days in study. There was an affect of diet ($P < 0.01$), wk ($P < 0.01$), but not a diet by wk interaction (P = NS) on ADG; Panel B. Final body weight (BW) (kg) There was an affect of diet ($P < 0.01$) wk ($P < 0.01$), and a diet by wk interaction ($P < 0.01$) on BW Panel C. Final hip height (HH) (cm). There was an affect of diet ($P < 0.01$), wk ($P < 0.01$), and a diet by wk interaction ($P < 0.01$) on Final HH. Final BW and HH were recorded preceding euthanasia. Superscripts represent differences between individual treatments across wk ($P < 0.05$).

Table 2-1. Nutrient composition of the LPN (low plane of nutrition) MR diet, HPN (high plane of nutrition) MR diet, and the calf starter grain. LPN MR = 20% CP and 20% fat, fed at 441g of DM/d; HPN MR = 27% CP and 10% fat, fed at 882 g of DM/d in week 1 and 1431 g of DM/d from weeks 2-8. Both diets were reconstituted to 15% milk powder [w/v].

Component	**MR**	**MR**	**Calf Starter**
	20/20: LPN	**27/10 HPN**	**Grain**
Monensm (mg/lb)			22.5
Diflubenzuron (mg/lb)	5.45		3
Lasalocid	36.0		
Crude Protein (% DM)	20.0	27.0	22.0
Crude Fat (% DM)	20.0	10.0	3.5
Crude Fiber (% DM)	0.15	0.15	8.0
Calcium (%)	0.75	0.75	1.4
Phosphorus (%)	0.70	0.70	0.45
Sodium (%)		1.30	
Selenium (ppm)			0.3
Vitamin A (IU/lb)	20,000	20,000	10,000
Vitamin D3 (IU/lb)	5,000	5,000	
Vitamin E (IU/lb)	100	150	

Table 2-2. Nutrient intake in Holstein bull calves fed LPN (low plane of nutrition) and HPN (high plane of nutrition) diets

	2 WK		4 WK		8 WK			P values		
Item	LPN (n = 4)	HPN (n = 5)	LPN (n = 4)	HPN (n = 4)	LPN (n = 5)	HPN (n = 5)	SEM	Diet	WK	Diet* WK
Milk replacer intake										
DM, g/d	390.80	844.09	398.83	995.18	411.27	1194.82	55.67	<0.01	<0.01	<0.01
CP, g/d	78.16	227.90	79.76	268.70	82.25	322.60	14.99	<0.01	<0.01	<0.05
Fat, g/d	78.16	84.41	79.77	99.52	82.25	119.48	5.65	<0.01	<0.01	<0.05
GE, Mcal/d	1.900	3.797	1.939	4.477	1.999	5.375	0.251	<0.01	<0.01	<0.05
Starter intake[1]										
DM, g/d	0.00	0.00	98.56	36.01	178.29	223.52	18.96	NS[2]	<0.01	<0.01
CP, g/d	0.00	0.00	24.64	9.00	44.57	55.87	4.74	NS[2]	<0.01	<0.01
Fat, g/d	0.00	0.00	3.94	1.44	7.13	8.94	0.76	NS[2]	<0.01	<0.01
GE, Mcal/d	0.000	0.000	0.4352	0.1590	0.7872	0.9869	0.0837	NS[2]	<0.01	<0.01
Total Intake[3]										
DM, g/d	390.80	844.09	497.39	1031.19	589.56	1418.34	56.52	<0.01	<0.01	<0.01
CP, g/d	78.16	227.90	104.40	277.70	126.82	378.47	15.17	<0.01	<0.01	<0.01
Fat, g/d	78.16	84.41	83.71	100.96	89.38	128.42	5.64	<0.01	<0.01	<0.05
GE, Mcal/d	1.900	3.797	2.374	4.636	2.786	6.362	0.254	<0.01	<0.01	<0.01

[1] Statistical analysis of starter intake compared 4 wk to 8 wk only

[2] Main effect of diet at 4 wk (P = 0.01)

[3] Total intake – starter plus MR

Table 2-3. Body composition (minus head) of bull calves fed LPN (low plane of nutrition) or HPN (high plane of nutrition) diets for 2, 4, and 8 wk. Body composition was determined using Dual Energy X-ray Absorptiometry (DEXA) immediately following euthanasia.

	2 WK		4 WK		8 WK		SEM	P values		
Item	LPN (n = 4)	HPN (n = 5)	LPN (n = 4)	HPN (n = 4)	LPN (n = 5)	HPN (n = 5)		Diet	WK	Diet*WK
SBW[1], kg	32.37	41.24	34.10	46.78	38.41	77.31	1.65	<0.01	<0.01	<0.01
Lean, kg	29.99	38.40	31.69	43.54	35.74	71.93	1.51	<0.01	<0.01	<0.01
Lean, %	92.693	93.106	92.938	93.098	93.030	93.040	0.147	NS	NS	NS
Fat, g	1249.75	1599.60	1320.25	1814.00	1489.40	3002.40	63.15	<0.01	<0.01	<0.01
Fat, %	3.8625	3.8800	3.8700	3.8775	3.8740	3.8840	0.0058	<0.05	NS	NS
BMC[2], g	1119.90	1243.40	1095.88	1427.90	1183.92	2379.30	103.12	<0.01	<0.01	<0.01
BMC, %	3.4425	3.0160	3.1900	3.0200	3.0900	3.0780	0.1526	NS	NS	NS
BMD[3], g/cm^2	0.8740	0.8898	0.8463	0.9193	0.8382	1.1018	0.0390	<0.01	<0.05	<0.05

[1]Scanned body weight (SBW) = lean + fat + BMC
[2]Bone mineral content (BMC)
[3]Bone mineral density (BMD)

Table 2-4. Organ weights of bull calves fed LPN (low plane of nutrition) or HPN (high plane of nutrition) at 2, 4, 8 wks. Organs were excised immediately following DEXA scanning. Organs were rinsed, dried, and weighed.

	2 WK		4 WK		8 WK					
Item	LPN (n = 4)	HPN (n = 5)	LPN (n = 4)	HPN (n = 4)	LPN (n = 5)	HPN (n = 5)	SEM	DIET	WK	DIET*WK
ECW[1], kg	27.800	34.168	28.467	37.632	30.544	62.564	1.087	<0.01	<0.01	<0.01
GI tract, g	4,361.0	7,007.8	6,908.3	10,364.3	11,941.2	17,360.0	1,085.2	<0.01	<0.01	NS
% ECW	15.7	20.5	24.1	27.1	39.2	27.7	2.9	NS	<0.01	NS
Liver, g	831.3	1,155.4	784.3	1,472.0	871.2	2,106.6	54.2	<0.01	<0.01	<0.01
% ECW	3.008	3.382	2.755	3.915	2.864	3.370	0.144	<0.01	NS	0.05
Heart, g	284.5	377.0	280.3	394.8	300.2	603.0	14.1	<0.01	<0.01	<0.01
% ECW	1.028	1.104	0.985	1.060	0.986	0.966	0.039	NS	NS	NS
Kidney, g	214.0	308.4	195.5	371.5	246.2	614.0	18.8	<0.01	<0.01	<0.01
% ECW	0.778	0.902	0.685	0.983	0.806	0.982	0.045	<0.01	NS	NS
Kidney[2], g	139.8	248.8	174.3	292.8	214.2	527.4	22.7	<0.01	<0.01	<0.01
% ECW	0.728	0.510	0.613	0.775	0.702	0.844	0.061	<0.05	NS	NS
Perirenal fat, g	23.0	53.8	19.8	73.8	26.8	77.2	7.9	<0.01	NS	NS
% ECW	0.080	0.158	0.070	0.193	0.090	0.124	0.020	<0.01	NS	NS
Lungs, g	878.8	826.8	705.3	964.0	790.8	1305.0	79.2	<0.01	<0.01	<0.01
% ECW	3.148	2.418	2.468	2.550	2.574	2.084	0.190	<0.01	NS	NS
Spleen, g	109.8	187.2	108.5	197.8	142.0	579.6	20.4	<0.01	<0.01	<0.01
% ECW	0.393	0.548	0.380	0.530	0.466	0.930	0.052	<0.01	<0.01	<0.01
Pancreas, g	17.8	33.6	20.8	24.5	25.6	42.0	4.0	<0.01	NS	NS
% ECW	0.0625	0.0960	0.0725	0.0625	0.0840	0.0680	0.0096	NS	NS	NS
Thymus, g	35.25	83.2	35.75	139.75	65.8	399.2	16.8	<0.01	<0.01	<0.01
% ECW	0.1275	0.2440	0.1275	0.3650	0.2160	0.6380	0.0349	<0.01	<0.01	<0.01

[1]Empty carcass weight (ECW)
[2]Perirenal fat removed

Chapter 3

The Role of Early Postnatal Nutrition and Estrogen Administration in Regulating Reproductive Tract and Uterine Gland Development in Holstein Dairy Calves

Introduction

Raising replacement heifers is the second largest expense to dairy producers after feed costs (Bailey, 2009). The long-term goals associated with rearing replacement heifers are obtaining maximal fertility rates, which then will maximize lifetime milk production. At the onset of puberty, heifers have the ability to be fertilized and reproduce successfully. Body weight greatly influences age at the onset of puberty; therefore, producers strive to feed heifers at a rapid growth trajectory to obtain puberty early (Funston et al., 2012). Feeding heifers a higher plane of nutrition during the preweaning growth period has been linked to reproductive success of calves later in life, such as decreased age at puberty and age at first calving (Rincker et al., 2011). Heifer calves that reach their reproductive potential earlier in their lifetime will be more reproductively successful and therefore, more profitable to producers.

Reproductive organs form during embryonic and fetal development however, they do not become completely functional until puberty is reached (Murashima et al., 2015). The uterus is unique because, unlike other reproductive organs, it does not fully develop in mammals until 2 to 3 months after birth (Spencer et al., 2012). Birth marks the beginning of adenogenesis, or uterine gland development, in mammals and concludes approximately 8 weeks later in sheep (Gray et al., 2001a). Adenogenesis involves differentiation of glandular epithelium from luminal epithelium followed by the coiling and branching of glandular epithelium throughout the stroma

(Gray et al., 2001a). Ontogeny of adenogenesis remains unclear in cattle (Cooke et al., 2013). In all mammals, glands synthesize, secrete, and transport enzymes, growth factors, cytokines, lymphokines, hormones, proteins, and other substances into the lumen of the uterus (Gray et al., 2001a). These secretions aid in conceptus and placental growth and fetal development and survival (Bazer, 1975). Without glands, the conceptus will not receive adequate nutrients, growth factors, and enzymes, etc., needed for growth, development, and survival (Gray et al., 2001b). In the absence of glands, fertilization and embryonic development occur but elongation and implantation of the conceptus are obstructed, leading to infertility (Gray et al., 2001b).

Although the initial activation of adenogenesis is ovary-, adrenal-, and steroid-independent in sheep (Spencer et al., 2012), there are several factors that can hasten, impede, or terminate adenogenesis throughout development (Gray et al., 2001a; Spencer et al., 2012). These factors include, but are not limited to: sex steroids (estrogens, progesterone, and their receptors) (Bartol et al., 1995; Carpenter et al., 2003a), growth factors (IGF, FGF, and HGF) (Spencer et al., 2005; Spencer et al., 2012), and other hormones (PRL and RLX) (Carpenter et al., 2003b; Chen et al., 2011). Estrogen and its influences on adenogenesis are species-specific (Cooke et al., 2013). Administration of an anti-estrogen at birth of piglets inhibited the occurrence of adenogenesis; conversely, administration at birth of an aromatase inhibitor, which prevents the conversion of testosterone to estrogen, had no impact on lamb adenogenesis (Carpenter et al., 2003a; Cooke et al., 2013). Knowing that early, preweaning diets can influence and hasten calf growth and development, we postulate that plane of nutrition in early life will also impact adenogenesis. However, no information exists on early, preweaning nutrition and its influence on postnatal reproductive development, specifically, adenogenesis in cattle and other ruminants.

The premise behind the following work was to gain an understanding of how preweaning nutrition could influence adenogenesis development and timeline of events in cattle. Also, we aimed to determine if administration of exogenous estradiol, after weaning of calves at 8 weeks, would alter adenogenesis, although it is assumed to be complete.

Materials and Methods

Experimental Design

Animal experiments were completed in accordance with and with the approval of the Virginia Tech Institutional Animal Care and Use Committee.

Two studies were completed. In study one, Holstein heifer calves were assigned randomly to a LPN diet (n=6) or a HPN diet (n=5). The LPN consisted of a MR containing 20% CP and 20% fat (Southern States Calf Maker MP; 20% CP and 20% fat; Richmond, VA) that was fed at 430 g of dry matter per day. The HPN diet consisted of 28% CP and 25% fat that was fed at 1,077 g of DM per day (Cow's Match, Jersey Blend; Land O'Lakes Animal Milk Products Co; Saint Paul, MN). Milk replacers were reconstituted to 15% [w/v] water and were fed twice daily until the end of wk 6. Beginning at wk 7, MR feedings occurred once daily, offering half of total amount per day. Calves were pair-fed starter grain (Southern States Intensity 22% Textured Calf Starter, Medicated) beginning at 4 wk. This was achieved by recording the amount of starter consumed by each HPN calf on one day, and offering the same amount of feed to assigned LPN calves on the following day. Calves were offered water *ad libitum* throughout the study. Weekly BW, HH, wither heights (WH), and heart girths (HG) were recorded. Twelve hours prior to euthanasia, calves were infused with bromodeoxyuridine (BrdU; Alfa Aesar, Ward Hill, MA) at 5 mg/kg BW. Calves were euthanized via a lethal dose of barbiturate (Beuthanasia-D, Merck Animal Health, Millsboro, DE; 11.1 mg/kg) at 8 wk of age.

In study two, Holstein heifer calves were assigned randomly to MR treatments listed above (n=12 LPN; n=11 HPN). Calves were fed MR once daily beginning at 7 wk and weaned completely at 8 wk. Calves were pair-fed starter grain throughout the study beginning at 4 wk and were offered *ad libitum* water. Weekly BW, HH, WH, and HG were recorded. Half of the calves in each group (n=6 LPN; n=5 HPN) were assigned randomly to receive estradiol implants (25.7 mg; Compusdose Implant) for 14 days beginning at the end of wk 7. It is indicated, per manufacturer, that each controlled release implant provides 120 pg/ml of estradiol for approximately 36 hours and 70-80 pg/ml thereafter for calves with the BW observed in this study. Plasma estradiol concentrations will be determined in future work. Calves were infused with 5 mg/kg of BW of BrdU 12 hours prior to euthanasia. Calves were then euthanized via a lethal dose of barbiturate (Beuthanasia-D, Merck Animal Health, Millsboro, DE; 11.1 mg/kg) at the end of 10 wk.

Reproductive Tract Harvest and Processing

Reproductive tracts were excised, trimmed of excess connective tissue and vaginal tissue and weighed. Thereafter, ovaries, oviducts, cervix and uterus were dissected and weighed. Number of visible follicles was counted on each ovary, and then ovaries were processed for future work (not included in this report). One ovary was snap frozen in liquid nitrogen and stored at -80 C. The remaining ovary was cut along its dorsal surface and fixed in 4% [w/v] paraformaldehyde.

Endometrial Histology

Uterine tissues were harvested for histology and RNA analysis. Two cross-sections (0.3 to 0.7 cm wide) from each uterine horn were collected, rinsed in PBS, and fixed in 4% paraformaldehyde. Cross sections were embedded in paraffin, sectioned at 5 μm, and 3 sections were mounted on each slide. Slides were deparaffinized and rehydrated in a graded alcohol

series. Following, slides were stained with Hematoxylin (Modified Harris: 3530) decolorized in 1.0% [v/v] acid ethanol solution (1 ml of Hydrochloric acid, 100 ml of 70% ethanol), dehydrated through alcohol and xylene, mounted, and a cover slip was applied. Four representative images in each of the two samples (n=8 images/heifer) were analyzed on Nikon Eclipse Ti microscope at 10x magnification. NIS Element Software (version 4.13) was used to calculate total area, number of glands, individual gland area, total gland area, and percent gland area within the intrastroma area (percent of total area occupied by glands). Averages of 5 apical to basolateral distances were taken to calculate luminal and glandular epithelial heights examined at 400x magnification.

Endometrial Sampling for RNA and Protein Analysis

Once uterine cross-sections were collected, endometrium was collected from the remainder of the uterus. The uterus was cut open along the antimesometrial edge of each horn. Several sections of endometria were collected throughout the length of both horns using scissors. Tissues were wrapped in aluminum foil and snap frozen in liquid nitrogen for future RNA and protein analysis.

Statistical Analysis

Data were analyzed by analysis of variance using the general linear model of the Statistical Analysis System (SAS institute, 1999). Dependent variables included diet for study 1 and diet, estrogen treatment and their interaction for study 2. Follicle number was analyzed both with and without using ovary weight as a covariate. Data presented as LSmeans and SEM. Significance was recorded as $P < 0.05$.

Results

Study 1: Effects of Plane of Nutrition on Reproductive Tract Development at 8 Weeks of Age

Calf Growth

The HPN calves had greater ($P < 0.01$) BW than LPN calves from wk 2 to 8 (Fig. 3-1). Similarly, ADG was greater in HPN calves than LPN calves from wk 1 to 8 (mean and SEM: 0.63 kg/d ± 0.03 versus 0.20 kg/d ± 0.03; respectively). Also, HH, WH, and HG were greater ($P < 0.01$) in HPL than LPN calves from wk 5 to 8 (data not shown) (Geiger et al., Abstract: 62881).

Reproductive Tract Weights and Follicle Numbers

Diet did not affect total reproductive weight (Table 3-1). Also, diet did not affect individual weight of the uterus, cervix and ovaries (Table 3-1). Follicle number was not affected by diet (Table 3-1), but the LPN calves contained greater ($P < 0.01$) follicle numbers when data were adjusted based on ovarian weight (Table 3-1).

Uterine Gland Development

Calves fed the HPN contained greater uterine gland numbers at 8 wk of age ($P < 0.05$) (Fig. 3-2). Also mean size of individual glands was smaller ($P < 0.05$) in HPN than LPN calves (Fig. 3-2). Median size of glands also was smaller ($P < 0.05$) in calves fed the HPN diet (data not shown). Diet did not affect luminal and glandular epithelial heights (Fig. 3-3).

Study 2: Effect of Plane of Nutrition and Estrogen Administration on Reproductive Tract Development at 10 weeks of age

Calf Growth

Calves fed the HPN diet for 10 wk had greater ($P < 0.01$) BW from wk 2 to 8 (Fig. 3-4). Average daily gain was greater for HPN calves independent of estradiol treatment from wk 1 to 8 (mean and SEM: 0.63 ± 0.03 versus 0.20 ± 0.03; respectively). Between wk 8 and 10, ADG was

similar across most treatments (mean and SEM: HPN without estradiol = 0.58 ± 1.0; HPN with estradiol = 0.68 ± 0.08; LPN without estradiol = 0.83 ± 0.8; LPN with estradiol = 0.93 ± 0.08); however, LPN estradiol-treated calves had lower ($P < 0.05$) ADG than HPN calves not given estradiol. Hip heights, WH, and HG were greater ($P<0.05$) for HPN calves beginning at 5 wk. Estrogen treatment did not affect HH, WH, and HG (Geiger et al., Abstract: 62881).

Reproductive Tract Weights and Follicle Numbers

Diet did not affect total reproductive tract weights (Fig. 3-5), once adjusted for BW but estradiol treatment increased ($P < 0.05$) reproductive tract weights (Fig. 3-5). Also, a diet by estradiol interaction existed ($P < 0.05$), where calves fed the HPN exhibited an increase in weights when estradiol was given whereas calves fed a LPN diet exhibited a decrease in tract weights when given estradiol.

Neither diet nor estradiol affected uterine weight. However, there was a diet by estradiol interaction ($P < 0.01$) where uterine weights were heavier for calves fed LPN without estradiol than calves fed LPN with estradiol. However, the inverse was seen in the HPN where calves not receiving estradiol had lighter uterine weights than calves receiving estradiol (Fig. 3-6). Cervix weight was not affected by diet but was affected by estradiol, with estradiol-treated calves in both diets having heavier cervices ($P < 0.05$) (Fig. 3-6). No diet by estradiol interaction was detected.

Diet and estradiol treatment did not affect ovarian weight, but there was a tendency ($P = 0.07$) for a diet by estradiol interaction (Fig 3-7). Estradiol treatment decreased ovarian weights for LPN fed calves and increased ovarian weights for HPN fed calves. There was no effect of diet on follicle number, but there was a tendency for estradiol treatment to increase ($P = 0.09$) follicle number in HPN calves (Fig. 3-7). No diet by estradiol interaction was detected. After normalizing for ovarian weight, diet did not affect follicle numbers, but calves in both diets with

estradiol contained greater ($P < 0.05$) follicle numbers than those that did not receive estradiol (Fig. 3-7).

Uterine Gland Development

Neither diet nor estradiol treatment affected total gland number, mean size of individual glands, nor percent gland area (percent of total area occupied by glands) (Fig. 3-8). Also, there were no diet by estradiol interactions detected (Fig. 3-8). Luminal epithelial heights were not impacted by diet but estradiol treatment increased ($P < 0.05$) luminal epithelial heights (Fig. 3-9). There was also a diet by estradiol interaction ($P < 0.05$) observed, where estradiol exposure increased ($P<0.05$) luminal epithelial heights in HPN fed calves (Fig. 3-9). LPN calves had greater ($P < 0.05$) glandular epithelial heights than HPN calves but no estradiol and diet by estradiol interaction was detected (Fig. 3-9).

Discussion

No information exists to explain how plane of nutrition and resulting early postnatal growth rates may impact reproductive tract parameters in dairy heifers. As anticipated, providing different milk replacer diets and pair-wise feeding starter grain succeeded in generating calves with different growth trajectories for the first 8 weeks (Brown et al., 2005; Sweeney et al., 2010). A clear deviation in BW, HH, WH and HG was evident at wk 8. It is evident that feeding a higher plane of nutrition can increase rate of gain, BW, HH, WH, and HG. By contrast, rates of gain and increases in BW, HH, WH, and HG were not detectable when all calves are offered an equal plane of nutrition between wk 8 and 10; in this case, solely a starter grain diet.

Though the importance of adequate nutrition for growth and development is widely recognized in calves, plane of nutrition and its impact on adenogenesis has not been described in cattle. The economic value of heifers to the dairy and beef industries limits terminal-based

studies, such as those completed herein. Therefore, the ovine model is the most widely studied ungulate for adenogenesis.

In the current study, plane of nutrition did not impact total reproductive tract growth and the weights of the uterus, cervix, and ovaries at 8 wk. However, underfeeding heifers during critical growth periods delayed the onset of estrus and compromises fertility (Otterby and Linn, 1981). Conversely, rapid growth of calves during periods of critical growth contributed to an earlier age of puberty and age at first calving (Rincker et al., 2011). Although diet did not impact reproductive tract weights, a clear correlation exists between nutrition and possible fertility.

Adenogenesis is a complex process governed by numerous steroids, growth factors, hormones, etc. Although the exact mechanisms of how those factors influence adenogenesis may not be fully understood, it is documented that they do in fact impact adenogenesis. However, nothing is known about early preweaning nutrition and if it influences adenogenesis. In this study, diet had no impact on intrastroma uterine area but HPN calves had a greater number of smaller glands and LPN had fewer, larger glands. Tubular glands begin coiling deep into the stroma from postnatal day (P) 21 to P56 prior to branching morphogenesis which marks the completion of adenogenesis in sheep at P56 (Gray et al., 2001a). These results suggest that HPN fed calves had progressive coiling and branching of the glands into the stroma of the uterine endometrium by 8 wk of age whereas the LPN calves were lagging behind in the extent of branching and coiling. Knowing that ovine adenogenesis is complete by P56 or 8 wks of age (Gray et al., 2001a), it is likely that a HPN fed to calves may hasten adenogenesis and LPN fed calves may be at a delayed stage of adenogenesis. In contrast, by 10 wks, diet had no impact on adenogenesis. At 10 wks, it can be assumed that calves withheld from nutrition had ample time

for adenogenesis to ‘catch-up’ and resume normal development. This is the only existing work recognizing diet and its influence on adenogenesis in mammals.

Another important aspect of this work was to assess the impact of estradiol treatment on reproductive tract development. The original work was preformed by Geiger et al., 2015 (Abstract: 62881) to evaluate the influence of preweaning nutrition and postweaning estradiol administration on mammary gland development. Their objective was to determine if preweaning nutrition altered the way the mammary glands respond to exogenous stimuli; specifically estradiol administration. The 8 wk study was completed to ensure that the diets used throughout the study would generate different growth trajectories. Eight wks was chosen as the end point in correlation with average weaning age of dairy heifers (NAHMS, 2007). The basis of the 10 wk study was to verify that exogenous estradiol over a 2-wk postweaning period would allow the mammary gland to respond differentially to estrogen at two different growth trajectories. The estradiol implants were given between wk 8 and 10 to detect any changes in mammary development when exogenous estradiol was given after weaning.

It was not surprising that estrogen implants did not impact growth rates in this work. Limit-feeding starter grain is one likely reason for this lack of effect. In a traditional industry setting, starter grain is offered *ad libitum* (Raeth-Knight et al., 2009), in such a case, a greater estradiol influence on growth performance can be anticipated. Another reason that estradiol may not have been effective at mediating growth rates was because of the short exposure time and early age of administration. Estrogen implants can benefit prepubertal heifer growth and development. Zeranol (estrogen agonist) implants administered at 3 months of age or later increased growth rates in heifers by 6.9%, increased average daily gain by 7% in heifers, and

improved feed efficiency by 6.1% in steers (Loy et al., 1988; Kirkwood et al., 1991; Moran et al., 1991).

Growth rates from wk 0 to 8 were similar between studies. After weaning, HPN fed calves without estradiol treatment experienced a decline or 'lag' in BW gain from 8 to 9 wk. This effect was short-lived, and calves continued on a rate of gain similar to that of other groups by wk 10. Reasons for this decline in BW are not clear. Weaning can cause a decline in BW gain (Khan et al., 2007b; Sweeney et al., 2010), and calves offered a HPN milk diet rely heavily on MR; and this could have compromised calf preparedness for starter grain intake (Sweeney et al., 2010). Interestingly, the HPN calves that did receive the estradiol treatment did not experience this delay in BW growth. These results are in agreement with other studies demonstrating estrogen's ability to improve gains, especially during weaning (Lammers et al., 1999).

There is minimal work that describes the effects of diet and estrogen on reproductive tract development. In 1983, Staigmiller et al. proposed that negative effects of estradiol or estradiol-like implants would be overcome by the beneficial influence of nutrition on fertility in yearling heifers (Staigmiller et al., 1983). Results of this study showed an increase in pelvic size of implanted heifers and no difference in pregnancy rate (Staigmiller et al., 1983). Deutscher et al. (1986) studied plane of nutrition and its interaction with zeranol from weaning to breeding. Pregnancy rate was decreased by 12% in implanted heifers maintained on a traditional nutrition level, but both implanted and control heifers fed a higher plane of nutrition had pregnancy rates of 92% (Deutscher et al., 1986). In the study presented herein, the estradiol implant did positively influence the overall weight of the reproductive tract and cervix weight. When calves were offered a LPN and estradiol implant, their reproductive tract and isolated uterine weights decreased, but HPN-fed calves would experience an increase in reproductive tract and isolated

uterine weights when estradiol was administered. These outcomes could be parallel to the previous weight parameter results seen in HPN calves with and without estradiol treatment, verifying estrogen's ability to improve growth and development.

There are conflicting outcomes about estrogen and its impact on reproductive development and productivity in cattle. As puberty approaches, the development of large dominant follicles promotes an increase in estrogen secretion. Prepubertal estrogen secretion is important for growth of reproductive organs such as, the uterus, cervix, and mammary glands (Bergfeld et al., 1994). Studies providing exogenous estrogen during the prepubertal phase have led to increased mammary development (Moran et al., 1991). However, exogenous estrogen during the preweaning and prepubertal phase can hinder reproductive tract development, delay the onset of puberty, and increase age at first calving (Lamb, 2013).

Our results provide evidence that exogenous administration of estradiol at the completion of adenogenesis has negligible affects on gland morphology and number. Administering estradiol at 8 wks of age for 2 wks did not influence adenogenesis in either dietary group. However, beginning exogenous steroid treatments in the early postnatal period compromises uterine gland development. Bartol et al. administered P4 and estradiol benzoate (EB), a synthetic E_2, to beef heifers at three different time points (P0, P21, or P45) to define the resulting impact on adult uterine morphology. Heifers treated with P4 and EB at all times experienced reduced uterocervical weights, myometrial area, and endometrial area. There was also a reduction in gland density by 65, 22, and 33% for treated heifers on P0, P21, or P45, respectively (Bartol et al., 1995). This proves that exogenous administration of hormones prior to the completion of adenogenesis will hinder or impede gland genesis.

Luminal and glandular epithelial height were not different in response to diet at 8 wks or 10 wks. However, when estradiol was introduced, calves offered a HPN had greatest luminal epithelial heights. This could relate to cows in estrus. At the time of estrus, when estrogen is at a peak, and a few days following, uterine luminal epithelium reaches a height of 20 μm (Marion and Gier, 1971). In contrast, Marinov and Lovell (1968) found disagreeing results, reporting luminal epithelium heights at an all time low during estrus and greatest 16 days post-estrus (Marion and Gier, 1971). It is evident that estradiol influences luminal epithelial heights but the reasoning for this interaction cannot be explained by this study.

The opposite was true for glandular epithelial heights. At 8 wks, diet had no influence of glandular epithelial heights and at 10 wks estrogen had no influence of glandular epithelial heights. However, diet impacted glandular epithelial heights at 10 wks. Calves offered a LPN acquired greatest glandular epithelial heights. This could be related to the results discussed concerning their delay in adenogenesis at 8 wks. Although the number, individual size, and average size of glands were not different between treatments at 10 wks, the differences in diet could have resulted in greater glandular epithelial content for LPN and a greater glandular lumen for HPN calves.

Diet had no influence on ovarian weight and follicle number at 8 or 10 wks. Estrogen increased follicle number. This could suggest that exogenous estradiol is indirectly influencing follicle selection or recruitment through the FSH feedback loop. Although it is known that follicular selection and recruitment require endogenous FSH and estrogen, the mechanism that regulates these actions is unknown in ruminants (Fortune et al., 1991).

These observations verify that diet and exogenous estrogen influence calf growth at 8 and 10 wks of age. Estrogen implants aid in minimizing weight loss due to weaning for HPN calves

at 10 wks. Results indicate that diet does not influence reproductive tracts weights at 8 or 10 wks; however, estradiol implants resulted in heavier reproductive tract weights and follicle number at 10 wks, similar to the BW results of HPN-fed calves at 10 wks. These observations conclude that bovine adenogenesis follows a similar timeline to ovine adenogenesis and the use of estradiol implants at the end of adenogenesis does not have influential impacts on gland morphology or development.

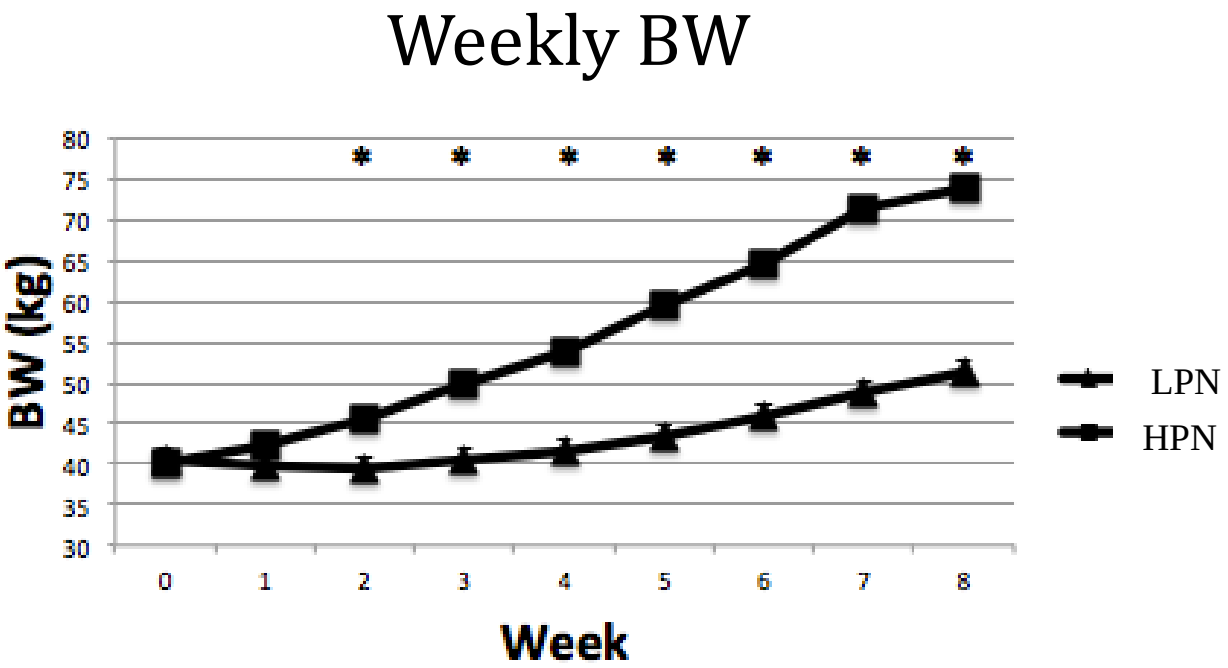

Figure 3-1. Weekly body weights (BW) from wk 0-8 of calves fed either LPN (low plane of nutrition) or HPN (high plane of nutrition) diets. Significance is shown by * from wk 2-8 ($P < 0.01$). Body weights were recorded once calves arrived on the farm and weekly thereafter. The asterisks (*) indicate differences in within-wk comparisons of each diet ($P < 0.05$). (Geiger et al. unpublished)

Table 3-1. Reproductive tract weights and follicle numbers of calves fed either a LPN (low plane of nutrition) or HPN (high plane of nutrition) diet at 8 wks. Reproductive tract weights were recorded after tissues were trimmed of excess connective tissue or fat, rinsed, and dried. (Weights are adjusted for BW) ($P < 0.05$)

	HPN (n=5)	**LPN (n=6)**	**P Value**
Reproductive Tract wt (g/kg BW)	**0.48**	**0.47**	**NS**
Uterus wt (g/kg BW)	**0.33**	**0.37**	**NS**
Cervix wt (g/kg BW)	**0.09**	**0.09**	**NS**
Ovary wt (g/kg BW)	**0.04**	**0.05**	**NS**
Follicle #	**31.0**	**30.7**	**NS**
Follicle # (#/g non-adj. ovary wt)	**10.3**	**14.2**	**0.01**

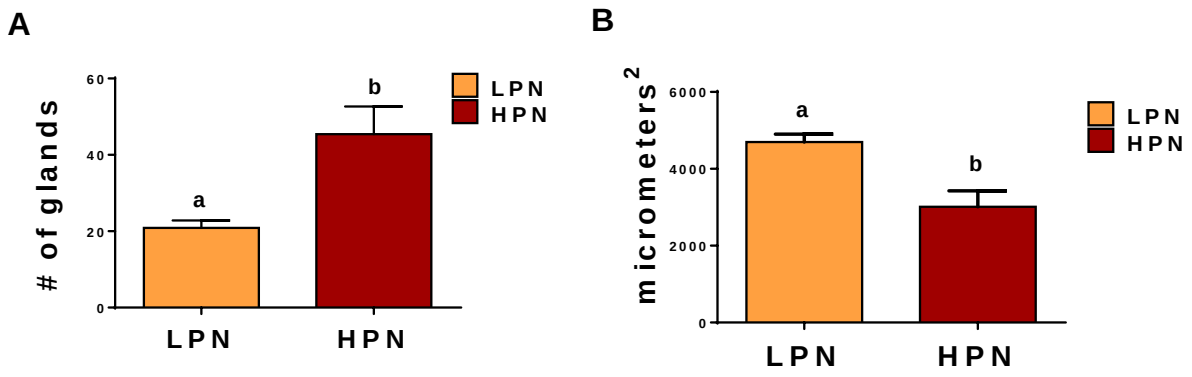

Figure 3-2. Diet and its influence on adenogenesis at 8 wk of calves fed either a LPN (low plane of nutrition) or HPN (high plane of nutrition). Panel A: Average number of glands (sum of visible glands within the intrastroma of each picture divided by the number of pictures counted) located within the image of intrastroma uterine area (n=8). Panel B: Average individual mean gland size. All glands within the image were traced and area was automatically calculated through NIS Element Software (version 4.13). Differing superscripts denote significant differences between diets. ($P < 0.05$)

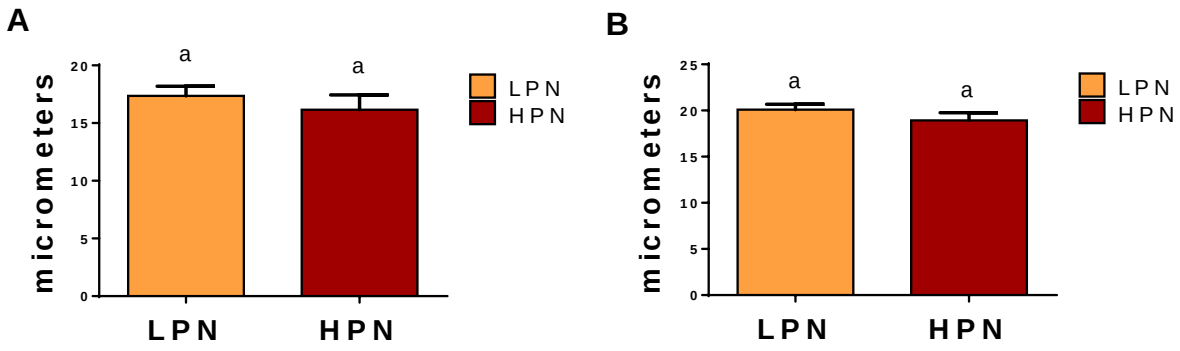

Figure 3-3. Diet and its influence on luminal and glandular epithelial heights at 8 wk of calves fed either a LPN (low plane of nutrition) or HPN (high plane of nutrition). Panel A: Average luminal epithelial heights (P = NS); Panel B: Glandular epithelial heights (P = NS); Luminal and glandular epithelial heights were determined by averaging 10 individual epithelial heights in each representative cross section using NIS Element Software (version 4.13). Pictures were taken with a Nikon Eclipse Ti microscope at 40x magnification.

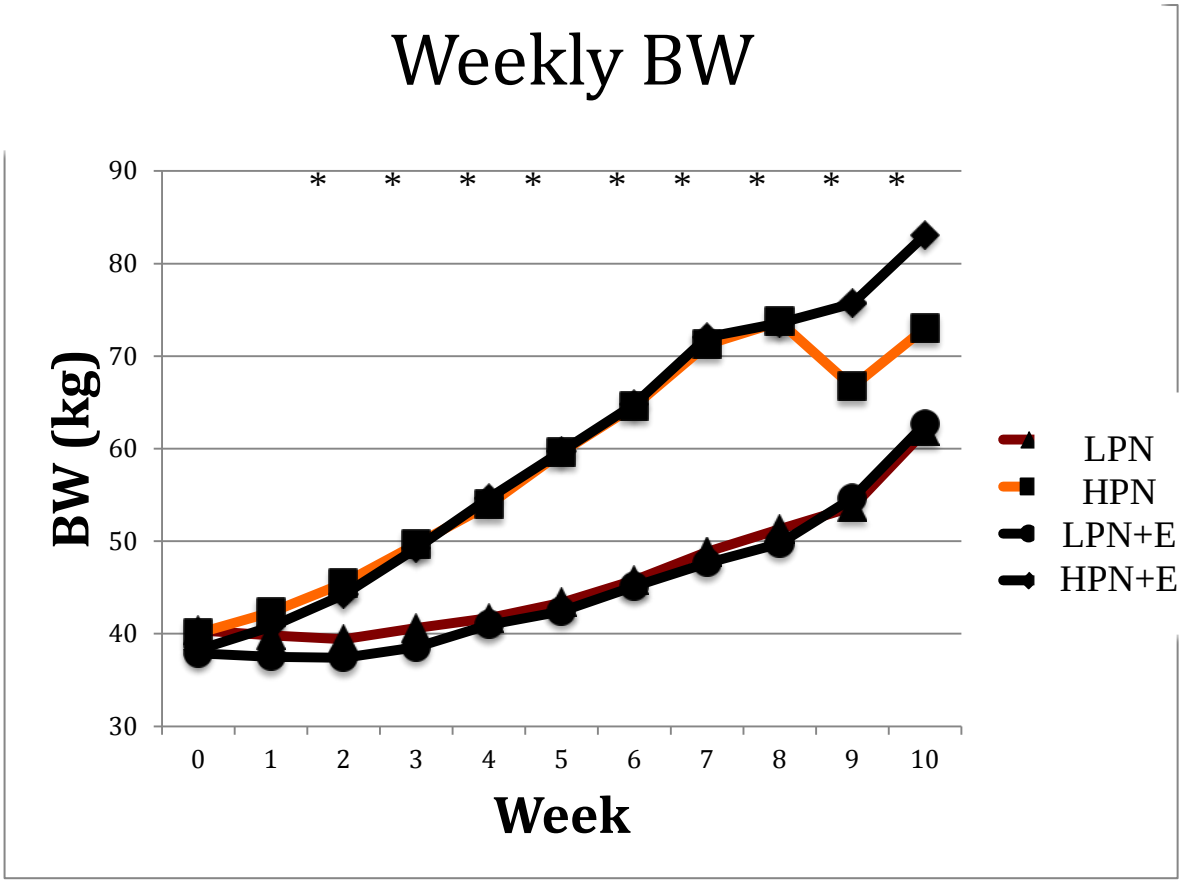

Figure 3-4. Weekly BW from wk 0-10 of calves fed either a LPN (low plane of nutrition) or HPN (high plane of nutrition) diet. The asterisks (*) indicate differences in within-wk comparisons of each diet ($P < 0.05$). (Geiger et al. unpublished)

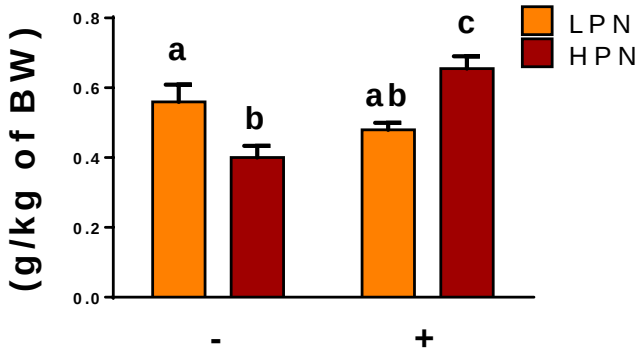

Figure 3-5. Reproductive tract weights of calves fed either LPN (low plane of nutrition) or HPN (high plane of nutrition) diet with (+) or without (-) estradiol treatment at wk 10. Reproductive tract weights were recorded after tissues were trimmed of excess connective tissue or fat, rinsed, and dried. Differing superscripts denote significant differences in a diet by estrogen interaction ($P < 0.05$). There was no influence of diet alone ($P = NS$), but there was an influence of estradiol ($P < 0.05$)

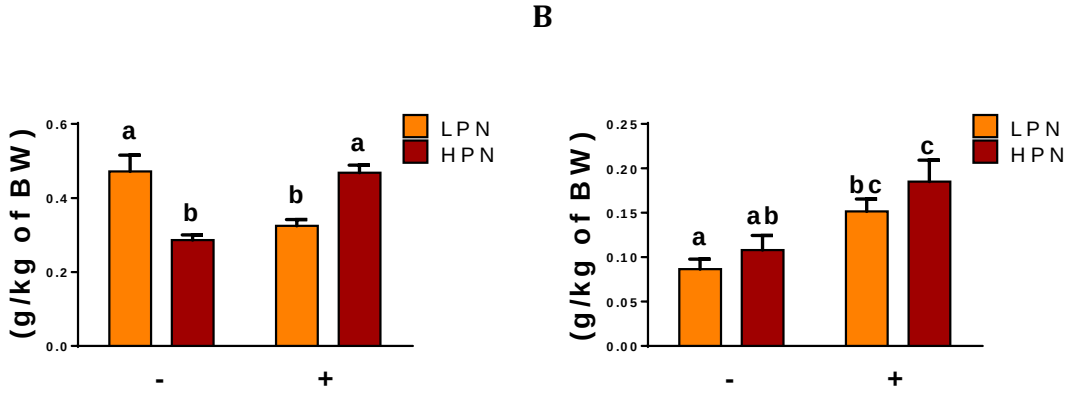

Figure 3-6. Uterine and cervical weights of heifer calves fed LPN (low plane of nutrition) or HPN (high plane of nutrition) with (+) or without (-) estradiol treatment at 10 wk. Panel A: Uterine weights, including uterine body and uterine horns. There was no influence of diet and estradiol alone (P = NS). Panel B: Cervix weights. There was no influence of diet (P = NS) however there was an estradiol influence (P < 0.05). Reproductive tract weights were recorded after tissues were trimmed of excess connective tissue or fat, rinsed, and dried. Differing superscripts denote significant differences of a diet by estradiol interaction (P < 0.05).

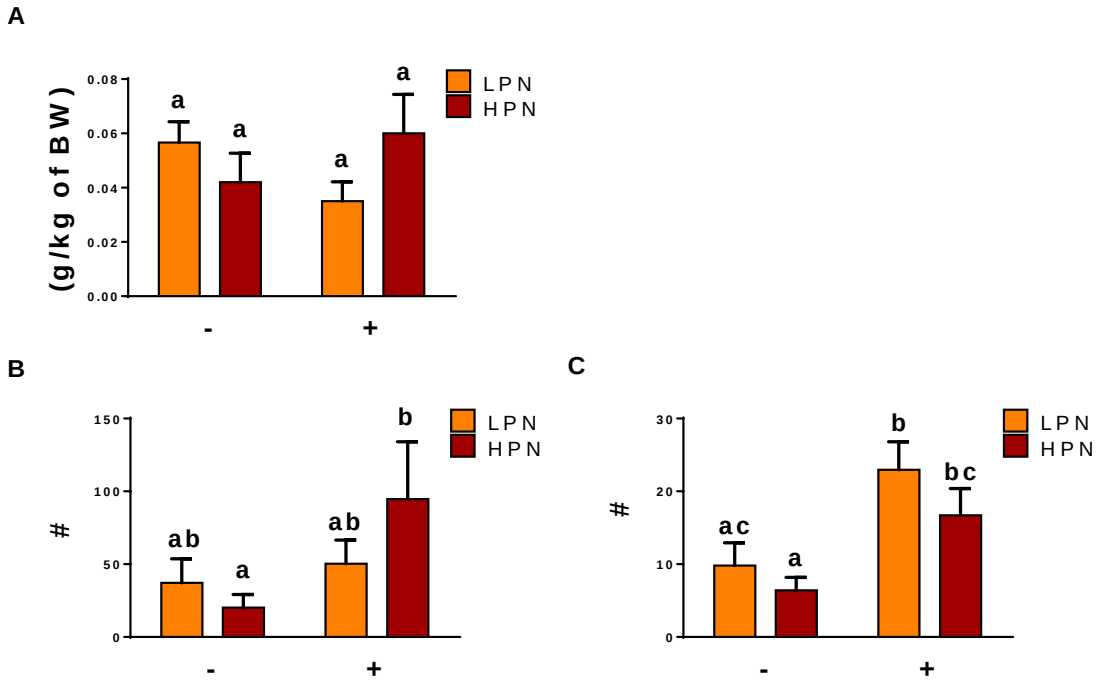

Figure 3-7. Ovarian weights and follicle numbers of heifer calves fed LPN (low plane of nutrition) or HPN (high plane of nutrition) with (+) or without (-) estradiol treatment, at 10 wk. Panel A: Sum of both ovarian weights. Ovarian weights were not influenced by diet or estradiol alone (P = NS). Panel B: Follicle number. Total visible follicles were counted on each ovary and summed together. Panel C: Follicle number adjusted to ovary weight. Total follicle number was determined using ovarian weight as a covariate. There was no influence of diet (P = NS) but there was an estradiol influence ($P < 0.05$) on follicle #. Differing superscripts denote significant differences of a diet by estradiol interaction ($P < 0.05$).

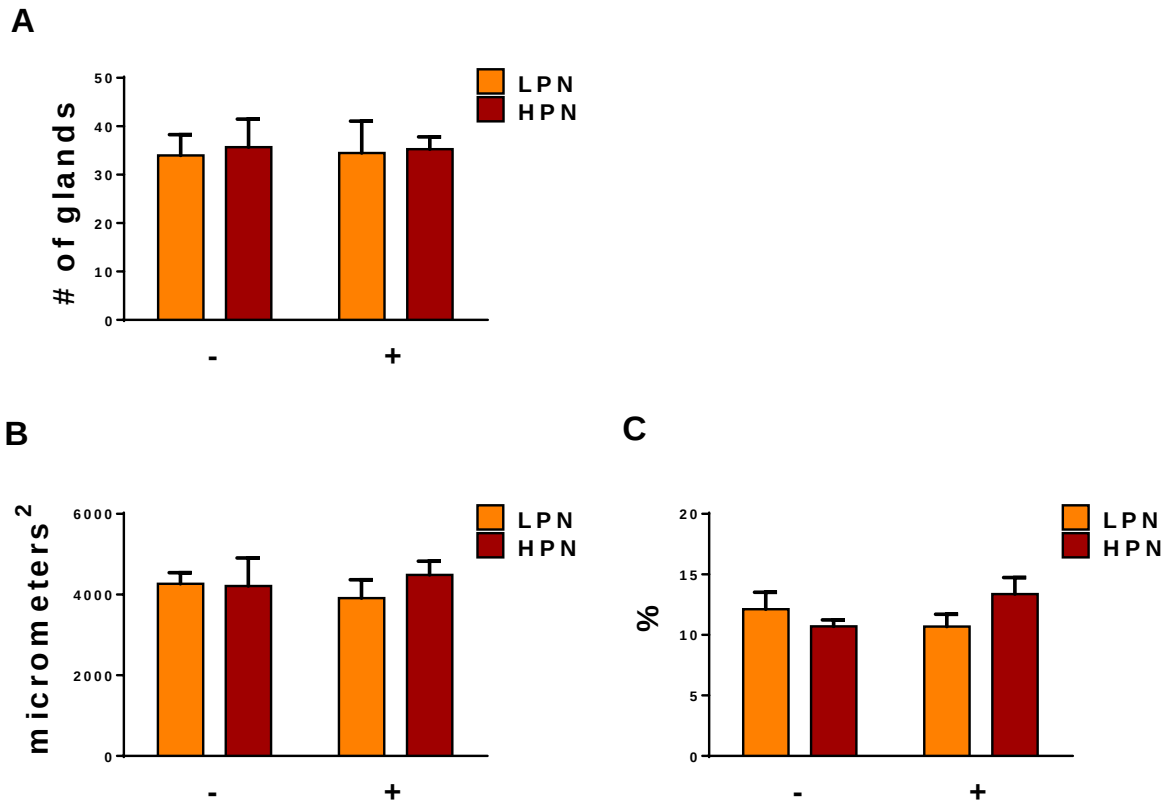

Figure 3-8. Analysis of adenogenesis of heifer calves fed LPN (low plane of nutrition) or HPN (high plane of nutrition) with (+) or without (-) estradiol treatment at 10 wk. Panel A: Average number of glands (sum of visible glands within intrastroma of each picture divided by the number of pictures counted) located within the image of intrastroma uterine area (n=8). Panel B: Individual, mean gland size (total sum of gland area in each image divided by the number of total glands). Panel C: Percent gland area (total gland area divided by the intrastroma uterine area analyzed). There was no influence on diet, estradiol, or a diet by estradiol interaction (P = NS) on gland parameters at 10 wks.

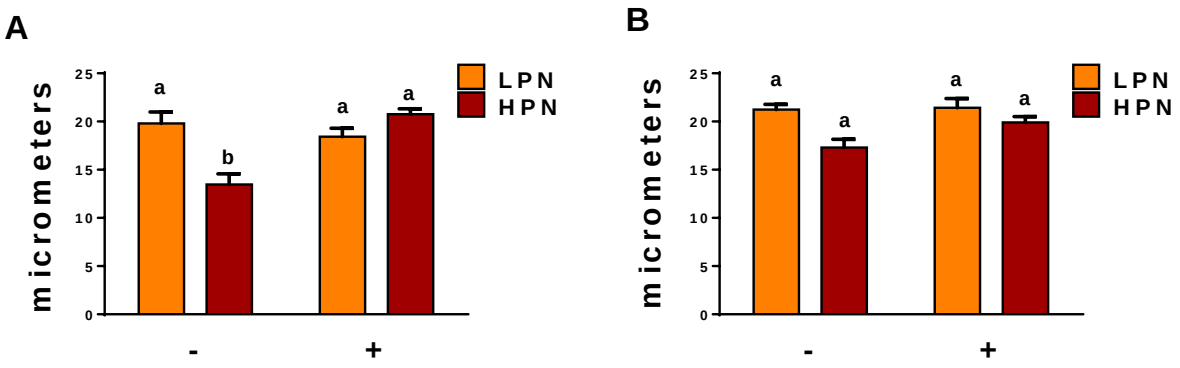

Figure 3-9. Luminal and glandular epithelial heights of heifer calves fed LPN (low plane of nutrition) or HPN (high plane of nutrition) with (+) or without (-) estradiol treatment at 10 wk. Panel A: Luminal epithelial heights. There was no influence of diet alone (P = NS), however there was an influence of estradiol ($P < 0.05$) on luminal epithelial heights. Panel B: Glandular epithelial heights. There was an influence of diet ($P < 0.05$), however there was no influence of estradiol on glandular epithelial heights. Luminal and glandular epithelial heights were determined by averaging 10 individual epithelial heights in each representative cross section using NIS Element Software (version 4.13). Pictures were taken with a Nikon Eclipse Ti microscope at 40x magnification. Differing superscripts denote significant differences across diet and treatment. ($P < 0.05$)

Interpretive Summary

During early postnatal development, calf growth is a strong predictor of potential health, development, and productive lifespan of a cow. Plane of nutrition directly correlates with onset of puberty. Reaching maximal growth rates during early development can hasten the onset of puberty, which therefore allows the calf to be reproductively efficient for a longer period of time. Raising replacement heifers comes to a large cost of producers. Controlling early calf growth and development through preweaning milk diets to accelerate reproductive potential provides economic benefits for producers.

The first study was performed to prove that early postnatal nutrition, specifically a milk diet, would easily influence calf growth and development of young calves. Results showed that calves offered a higher plane of nutrition versus a lower plane of nutrition through milk replacer had higher average daily gains, greater feed efficiencies, and more lean muscle deposition. Other observations included greater bone mineral density and heavier organs, specifically immune-centric organs at time of slaughter. These beneficial effects of diet on bone mineral density and immune-centric organ growth indicate the importance of nutrition prior to weaning and may provide new possibilities for understanding the impacts of early nutrition on calf immune response development and lifetime productivity of the cow. Using a similar model in future studies could be beneficial if neutrophil activation rate and lymphocyte presence was analyzed to determine the exact immune responses or immune processes occurring within each animal across treatment. Understanding the exact influence that the preweaning diet has on immune response development may help to lower mortality and morbidity rates of young calves during early development.

The second study focused primarily on nutrition and exogenous estradiol administration and its influence on reproductive development and adenogenesis in Holstein heifer calves. Results indicate that preweaning diet has no influence on reproductive tract weights at 8 or 10 wks of age. Currently, no work exists on nutrition and its influence on adenogenesis. Also, very little work exists examining the timeline of adenogenesis in the bovine model.

It has been assumed that bovine adenogenesis occurs at a similar rate of development of ovine adenogenesis and is complete at the same time, 56 d or 8 wks. Results indicate that preweaning diets positively influence adenogenesis. Calves offered a higher plane of nutrition had greater number of glands at a smaller diameter than calves offered a lower plane of nutrition. This may suggest that diet can hasten gland development and calves fed for accelerated growth had greater coiling and branching morphogenesis by 8 wk of age compared to calves fed a lower plane of nutrition. Also, these results prove that adenogenesis in the bovine model is complete by 56 d or 8 wks when calves are offered adequate nutrition. However, there was no difference in gland formation or number at 10 wk due to diet. This suggests that calves offered a lower plane of nutrition during the preweaning period needed 2 additional weeks of development to 'catch-up' to accelerated fed calves. Estradiol administration post- completion of adenogenesis influence reproductive tract weights but made no impression on adenogenesis at 10 wk. We can assume that administration of exogenous estradiol administered at the completion of gland development does not hasten or hinder adenogenesis. Better understanding the timeline of bovine adenogenesis emphasizes the importance of preweaning nutrition of dairy calves. Future studies involving nutrition and reproductive organ development will be beneficial for understanding the productive lifespan of the cow. Collectively, these studies emphasize the benefits of manipulating early growth and development through preweaning milk diets and provide insight

of the underlying mechanisms controlling or influencing the onset of puberty.

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